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THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, EXTRACTION WITH DILUTE ACID SOLUTIONS, AND CROP RESPONSE TO FERTILIZATION

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Although electrodialysis has been used by many different investigators, to study base exchange reactions in soil, very little information is available concerning the solubility of soil phosphates under the influence of the electric current and crop response to phosphorus fertilization. Cameron and Bell (4) studied the rate of solution of phosphorus in soil, using 5 gm. of soil and a 40-volt current, and found that phosphorus was slowly removed from soil by such treatment.

König, Hasenbaumer, and Glenk (7) studied the diffusion of phosphorus from soil placed on a parchment membrane and found that electrodialysis hastened the rate of removal of the phosphorus. Mattson (9) analyzed the dialyzate from certain soil colloids and found varying amounts of phosphorus in the cathode compartment. Rost (10) reports that electrodialysis is more important in detecting lime deficiency in soils than phosphorus deficiency. Only eight soils were studied. Humfeld (6) found that soil to which limestone, rock phosphate, and superphosphate had been added contained more easily soluble phosphorus, as determined by electrodialysis, than did untreated soil. Alben (1) studied the amount of phosphorus removed from two soils by electrodialysis and concluded that no correlation existed between the results obtained and the crop response from phosphorus fertilization. McGeorge and Breazeale (8) found that the removal of phosphorus from calcareous soils by electrodialysis correlated very well with phosphorus deficiency in one group of soils but did not correlate in a second group of soils where phosphorus availability was assumed to be the limiting factor in crop production.

If different kinds of phosphates exist in a soil and vary in their rate of solubility, electrodialysis should make it possible to study the rate of solution of soil phosphorus because the solute is quickly removed from the water film surrounding the soil particle and as a result more material will continue to go from the solid to the liquid phase. In previous investigations the number of samples which have been studied are hardly sufficient to give a very good index concerning the relation between the amount of phosphorus removed from soil by electrodialysis and response to phosphorus fertilization. Also it is quite possible that the time factor, or the voltage, or the amount of current which flows through the soil may have some effect on the results.

EXPERIMENTAL

In the following investigation several different phosphate minerals and a large number of soils were studied in order to determine whether or not electrodialysis could be used in measuring the rate of availability of soil phosphorus to plants. A comparison was made between the amount of phosphorus removed from soil by electrodialysis; the amount of available phosphorus as determined by the Bray method (3), the Truog method (11), and extraction with fifth normal sulfuric acid (5); and the response of crops to phosphorus fertilization.

The procedure which was used in the electrodialysis of the soil samples was as follows:

Ten grams of soil were placed between two strips of parchment paper in a cell similar to the one described by Mattson (9). The electrodes were made of platinum gauze and were approximately 2½ inches square. A current of 220 volts, regulated by placing a 50-watt lamp in the circuit, was used. One electrode was placed on each side of the soil sample, 200 cc. of distilled water was added to each side of the cell, and electrodialysis was continued for 3 hours. The solutions were removed from each cell at the end of the first 3-hour period, a second portion of distilled water was added to each compartment, and the dialysis was continued for a second 3-hour period. The solutions were changed against the end of the second 3-hour period and dialysis continued for a third period of approximately 15 hours. One hundred and fifty cubic centimeters of distilled water was placed in the anode chamber and 250 cc. of distilled water was placed in the cathode chamber at the beginning of the third period, since there was a gradual flow of water into the anode chamber as a result of electro-endosmosis and over a period of 15 hours a change of about 50 cc. occurred in these experiments. The solution which was removed from the cathode compartment was placed in a 250-cc. beaker, 5 cc. of concentrated nitric acid added, and the liquid evaporated to dryness. The residue was treated with 5 cc. of concentrated nitric acid and digested on a hot plate to destroy organic matter, which interferes with the colorimetric determination of phosphorus. This process was repeated if necessary. Although this treatment may be considered objectionable from the standpoint of measuring accurately the amount of inorganic phosphorus present in solution, it is well known that traces of organic acids will prevent or at least decrease the color produced in the molybdenum blue method for phosphorus; consequently the aforementioned procedure was used, since the error from organic phosphates should tend to approach a constant value and would not be as serious as a failure to obtain a test for inorganic phosphorus although it was present in the solution.

After the organic matter was removed, 5 to 10 cc. of concentrated nitric acid was added and the solution was digested at 90°C. for 10 to 15 minutes. Then 25 cc. of distilled water was added and digestion continued for 15 minutes. The solution was cooled, 5 drops of 0.1 per cent paranitrophenol was added, and the excess acid was neutralized with 1 to 1 ammonium hydroxide until a yellow color appeared. Then it was made slightly acid by the careful addition of nitric acid, containing 1 part of acid and 3 parts of water, the disappearance of the yellow color being used as an end point in the titration. After the solution was acidified it was made up to a volume of 100 cc. and an aliquot was removed for a phosphorus determination. The molybdenum blue method was used to determine the phosphorus when small amounts were present and the standard volumetric method was used when accurate results could not be obtained by the colorimetric method.

The Truog method for easily soluble phosphorus was used as recommended except that the sulfuric acid was not buffered to pH 3, since in most cases the pH of the filtrates varied from 3.0 to 5.6 after extraction. Further investigation also indicated that buffering the solution with potassium sulfate did not appreciably affect the amount of phosphorus removed from the soil.

The Bray method for available phosphorus was used as recommended.

The determination of the easily soluble phosphorus in soils by extraction with fifth normal sulfuric acid has been used in this laboratory for several years and has given very good results under Oklahoma conditions. Ten grams of soil is shaken intermittently with 100 cc. of fifth normal sulfuric acid for 20 minutes. The suspension is filtered and an aliquot, usually 5 cc., is diluted to 95 cc. with

distilled water. Then the phosphorus is determined by the molybdenum blue method as recommended by Atkins (2).

RESULTS OF INVESTIGATIONS

In order to determine the relation between the rate of solubility of phosphorus in different phosphate minerals, several different samples were obtained and the solubility of the phosphorus in these samples was determined by electrodialysis, 0.5 gm. of each mineral, which was ground to pass through a 100-mesh sieve, being used. Each sample was mixed thoroughly with 9.5 gm. of quartz sand and was dialyzed for three successive periods as explained under methods for the electrodialysis of soil. The results of this experiment are given in table 1.

TABLE 1

Rate of removal of phosphorus from mineral phosphates by electrodialysis

NUMBER	MINERAL	CHEMICAL FORMULA*	TOTAL PHOSPHORUS	TOTAL PHOSPHORUS REMOVED BY ELECTRODIALYSIS		
				3 hours	6 hours	21 hours
			per cent	*per cent*	*per cent*	*per cent*
1	Apatite	$Ca_3(PO_4)_2F$	16 41	10 14	20.17	41 37
2	Chlorapatite	$Ca_3(PO_4)_2Cl$	15 60	25 44	39 16	72 24
3	Collinsite	$(Ca_2(Mg,Fe)(PO_4)_2 \cdot 2\frac{1}{2}H_2O$	13 93	18.45	38.19	76 68
4	Collophanite	$Ca_3(PO_4)_2 \cdot H_2O$	13 50	12 94	30 34	71 19
5	Dufrenite	$FePO_4 \cdot Fe(OH)_3$	2 54	7 40	11 41	14 64
6	Griphite	$(Mn,Ca,Na_2)O \cdot Al_2(PO_4)_2 \cdot H_2O$	14 42	15 73	35 83	71.31
7	Quercyite	$Ca_3(PO_4)_2 \cdot x\ H_2O$	14 31	9 84	24 29	59 19
8	Triplite	$(Mn,Fe)PO_4 \cdot F$†	11 34	8 81	20 08	45.46
9	Variscite	$AlPO_4 \cdot 2H_2O$	14 63	3 00	5 67	8 60
10	Vivianite	$Fe_3(PO_4)_2 \cdot 8H_2O$	12 31	1 54	2 10	3.89
11	Wavellite	$Al_3(OH)_3(PO_4)_2 \cdot 4\frac{1}{2}H_2O$	9 45	1 53	3 12	5.85

* Minerals were obtained from Ward's Natural Science Establishment, Rochester, New York.

† Some Ca and Mg usually present.

The amount of phosphorus removed from chlorapatite, collinsite, and collophanite, which are calcium phosphate minerals, was much higher than the amount of phosphorus removed from the iron and aluminum phosphates, dufrenite, variscite, vivianite, and wavellite. Three samples were in an intermediate group. They were apatite, quercyite, and triplite. The quercyite contained much organic matter, which may have accounted for the low results with this particular mineral.

If certain soils contained the major portion of their phosphorus in the form of iron or aluminum phosphate, less phosphorus should be extracted from these soils than from soils which contained a major portion of their phosphorus in the form of calcium or magnesium phosphate. Also if a soil contained only a small portion of its available phosphorus in the form of calcium phosphate, it

TABLE 2

Source of soil samples used in this investigation

SOIL NUMBER	LOCATION	EXPERIMENT OR PLOT NUMBER
69	Sapulpa, Okla.	Cotton Fertility Study, Outfield Station
93	Poteau, Okla.	Cotton Fertility Study, County Fair Ground
95	Chandler, Okla.	Sweet Clover Experiment, Frank Carpenter Farm
102	Durant, Okla.	Cotton Fertility Study, Outfield Station
103	Purcell, Okla.	Cotton Fertility Study, Outfield Station
106	Eufaula, Okla.	Rotation Experiments, Outfield Station
118	Stillwater, Okla.	Plot 6205, Experiment Station Farm
136	Stillwater, Okla.	Manured Wheat, Field "O"
137	Stillwater, Okla.	Unmanured Wheat, Field "O"
210	Tifton, Ga.	Cotton Fertility Experiment
334	Purcell, Okla.	Cotton Fertility Experiment, H. E. Perkinson Farm
348	Harrah, Okla.	Cotton Fertility Experiment, W. M. Pace Farm
445	Orlando, Okla.	Alfalfa Fertility Experiment, W. D. Thedford Farm
447	Ralston, Okla.	Alfalfa Fertility Experiment, J. W. Chase Farm
449	Statesville, N. C.	Plot 5, Field B, Fertilizer Experiment on Cotton
450	Stillwater, Okla.	Lewis Field Cotton Experiment, 1927
452	Stigler, Okla.	Sweet Clover Experiment, Blankenship Farm
470	Shawnee, Okla.	Cotton Fertility Experiment, John Price (tenant)
477	Kingston, R. I.	Plot 67N, R. I. Agr. Exp. Station
478	Lincoln, Neb.	Plot 442, Neb. Agr. Exp. Station
479	Scotts Bluff, Neb.	Plot 3411, Scotts Bluff Agr. Exp. Station
482	Scotts, Ark.	Fertility Experiments, Arkansas Agr. Exp. Substation
483	Fayetteville, Ark.	Sect. 24, Rotation Experiments, Arkansas Agr. Exp. Sta.
486	Raleigh, N. C.	Cecil Clay Loam, N. C. Agr. Exp. Station
487	Greenville, Ky.	Plot 310, Kentucky Agr. Exp. Substation
488	Lexington, Ky.	Plot 203, Kentucky Agr. Exp. Station
489	Beeville, Texas	T 31–40, Texas Agr. Exp. Substation No. 1
490	Angleton, Texas	Lake Charles Clay, Texas Agr. Exp. Substation
491	College Station, Texas	Plot 43, Texas Agr. Exp. Station
492	Paulding, Ohio	Plot 7, Block C, Rotation I, Ohio Agr. Exp. Substation
493	Wooster, Ohio	Plot 10 W, Section D, 5-yr. Rotation, Ohio Agr. Exp. Station
498	Joliet, Ill.	Plot 407, Ill. Agr. Exp. Field Station
500	Columbia, Mo.	Plot 9, Sanborn Field, Mo. Agr. Exp. Station
501	Manhattan, Kans.	Plot 2, Series 2, Kan. Agr. Exp. Station
503	New Brunswick, N. J.	Plot 7 A. N. J. Agr. Exp. Station
504	Bedford, Ind.	Indiana Agr. Exp. Substation
505	Lafayette, Ind.	Indiana Agr. Exp. Substation
506	Hennessey, Okla.	Wheat Fertility Experiment, Art. Ulmark Farm
507	Fairmont, Okla.	Wheat Fertility Experiment, Clarence Niehus Farm
513	Stigler, Okla.	Fertility Experiments on Corn and Cotton, P. E. McDaniel Farm
616	Perry, Okla.	Alfalfa Fertility Experiment, J. H. Dolezal Farm
663	Carrier, Okla.	Wheat Fertility Experiment, A. E. Ford Farm
703	Keosanqua, Ia.	Plot 1, Outfield Experiments, Iowa Agr. Exp. Station
707	Waverly, Iowa	Field No. II, Plot 1, Outfield Exps. Ia. Agr. Exp. Station
708	Calamus, Ia.	Plot 10, Outfield Experiments, Ia. Agr. Exp. Station

TABLE 2—*Concluded*

SOIL NUMBER	LOCATION	EXPERIMENT OR PLOT NUMBER
712	Ames, Iowa	Plot 1310, Iowa Agr. Exp. Station
712	Ames, Iowa	Plot 1000, Iowa Agr. Exp. Station
722	Granite, Okla.	Wheat Fertility, Exp. Plot 10, Okla. Agr. Exp. Substation
732	Glencoe, Okla.	Sweet Clover Fertility Experiment, J. Waltermire Farm
733	Columbus, Kans.	Plot 1 F, Kansas Agr. Exp. Substation
735	Moran, Kans.	Plot 1 A, Kansas Agr. Exp. Substation
737	Lenapah, Okla.	Pasture Fertility Experiment, Norman Mounce Farm
749	Granite, Okla.	Cotton Fertility Experiment, S. W. Parr Farm
750	Granite, Okla.	Cotton Fertility Experiment, Okla. Agr. Exp. Substation
754	Drumright, Okla.	Alfalfa Fertility Experiment, James Salisbury Farm
762	Stillwater, Okla.	Alfalfa Fertility Experiment, Range 1110 Okla. Agr. Exp. Sta.
790	Stillwater, Okla.	Alfalfa Fertility Experiment, Snowden Farm
797	Jennings, Okla.	Alfalfa Fertility Experiment, Carl Harbison Farm
858	Lovell, Okla.	Wheat Fertility Exp., C. L. Terhune Farm
862	Garber, Okla.	Wheat Fertility Exp., Rollie Kingery Farm
865	Waukomis, Okla.	Wheat Fertility Exp., Oscar Winchester Farm
894	Neodesha, Okla.	Fertility Experiments, W. O. Rittenhouse Farm
927	Goodwell, Okla.	Panhandle Agr. Exp. Station
962	Marianna, Ark.	Arkansas Agr. Exp. Substation
992	Union, Okla.	Fertility Experiments, Joseph Roy Farm
1032	Mulhall, Okla.	Cotton Fertility Experiments, Lincoln Lewis Farm
1044	Coweta, Okla.	Fertility Experiments, T. R. Stone Farm
P 1	Deer Creek, Okla.	Sweet Clover Fertility Experiment, M. C. Lichti Farm
P 8	Perry, Okla.	Sweet Clover Fertility Experiment, F. J. Blecha Farm
P 15	Perry, Okla.	Sweet Clover Fertility Experiment, C. J. Wallerstedt Farm
P 29	Deer Creek, Okla.	Sweet Clover Fertility Experiment, Emil Dester Farm
P 36	Chandler, Okla.	Sweet Clover Fertility Experiment, Lincoln County Farm
P 43	Okemah, Okla.	Sweet Clover Fertility Experiment, V. W. Miracle Farm
P 50	Perkins, Okla.	Sweet Clover Fertility Experiment, George Kirk Farm
P 57	Morris, Okla.	Sweet Clover Fertility Experiment, Morton & Hathaway Farm
P 64	Checotah, Okla.	Sweet Clover Fertility Experiment, Roy Nichols Farm
P 69	Mulhall, Okla.	Sweet Clover Fertility Experiment, Cleve Martin Farm
P 76	Neodesha, Okla.	Sweet Clover Fertility Experiment, W. O. Rittenhouse Farm
P 83	Beggs, Okla.	Sweet Clover Fertility Experiment, F. E. Wilson Farm
P 90	Guthrie, Okla.	Sweet Clover Fertility Experiment, H. L. Morrissett Farm
P 206	Duncan, Okla.	Sweet Clover Fertility Experiment, L. A. Morton Farm
P 216	Pawnee, Okla.	Sweet Clover Fertility Experiment, Stanley Dugan Farm
P 222	Pawnee, Okla.	Sweet Clover Fertility Experiment, L. F. Crocker Farm

would be removed in a short time and then the amount of phosphorus extracted would decrease very rapidly as the electrodialysis was continued.

In order to determine the variation in the amount of easily soluble phosphorus extracted from different soils, 83 different samples of soil were studied. These soils were obtained from unfertilized plots from fertility experiments, conducted in Oklahoma and in several other different places in the United States. The location from which each sample was obtained and the plot number or nature of the experiment are given in table 2. All of the samples in table 2 were analyzed for available phosphorus by electrodialysis, three successive extractions, as explained under methods of analysis, being used. Since the results from these extractions indicated that soils contain a certain portion of their phosphorus which is present in a readily available form, only the data

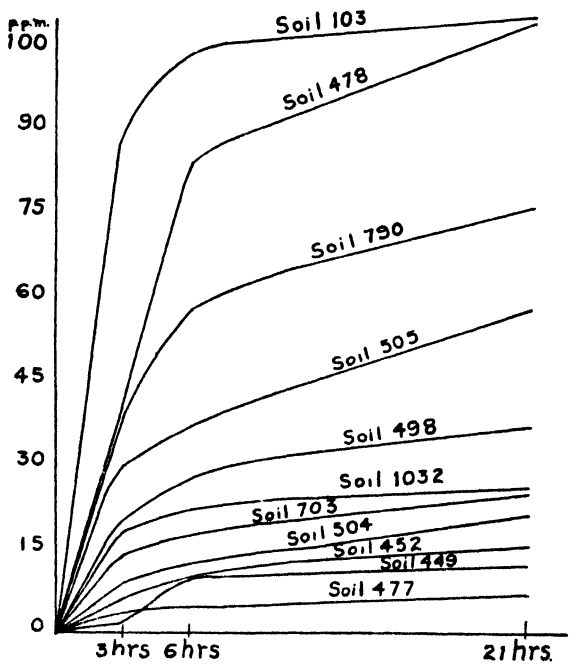

FIG. 1. SOLUBILITY CURVES OF SOIL PHOSPHORUS EXTRACTED BY ELECTRODIALYSIS

from a few representative samples are presented in figure 1. In all cases except one the amount of phosphorus extracted by electrodialysis during the first 3-hour period is larger than the amount extracted during the second and third 3-hour period. Soil 449 yielded more phosphorus in the second 3-hour period than it did in the first 3-hour period. However the amount of phosphorus removed from this soil was very low and it would have no influence on the recommendation which would be made concerning the need of this particular soil for phosphorus fertilization.

The comparison between the amount of phosphorus extracted by electrodialysis for a 3-hour period and the amount of phosphorus removed by three

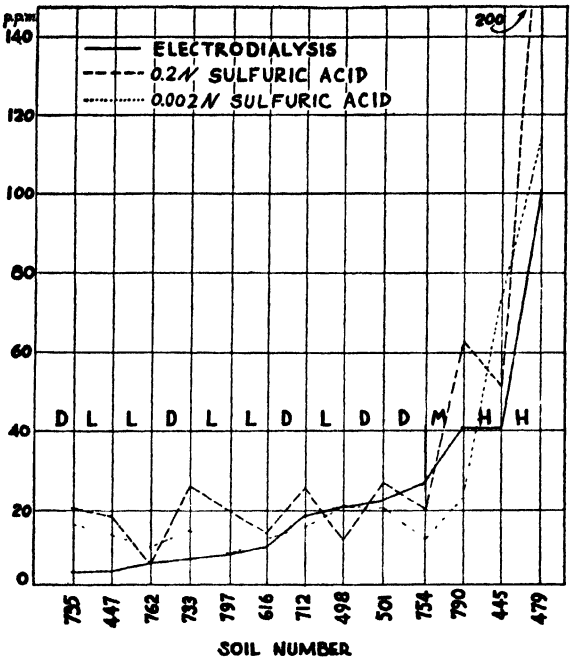

FIG. 2. STUDIES ON THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, SULFURIC ACID EXTRACTION, AND THE BRAY TEST, AS COMPARED WITH THE RESPONSE FROM FIELD EXPLRIMENTS WITH PHOSPHATE FERTILIZERS APPLIED TO ALFALFA

The first 10 soils beginning at the left respond to phosphorus fertilizatioL

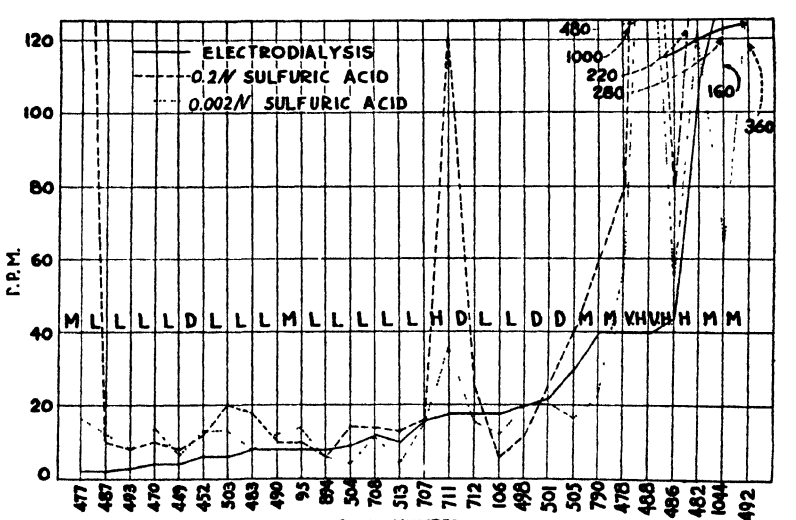

FIG. 3. STUDIES ON THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, SULFURIC ACID EXTRACTION AND THE BRAY TEST, AS COMPARED WITH THE RESPONSE FROM FIELD EXPERIMENTS WITH PHOSPHATE FERTILIZERS APPLIED TO CORN

The first 19 soils beginning at the left respond to phosphorus fertilization

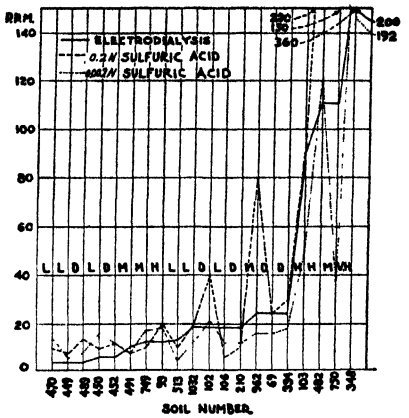

FIG. 4

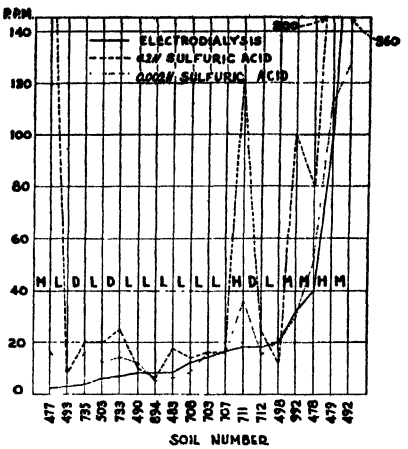

FIG. 5

FIG. 4. STUDIES ON THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, SULFURIC ACID EXTRACTION, AND THE BRAY TEST, AS COMPARED WITH THE RESPONSE FROM FIELD EXPERIMENTS WITH PHOSPHATE FERTILIZERS APPLIED TO COTTON

The first 13 soils beginning at the left, except soil 491, respond to phosphorus fertilization

FIG. 5. STUDIES ON THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, SULFURIC ACID EXTRACTION, AND THE BRAY TEST, AS COMPARED WITH THE RESPONSE FROM FIELD EXPERIMENTS WITH PHOSPHATE FERTILIZERS APPLIED TO OATS

The first 14 soils beginning at the left respond to phosphorus fertilization

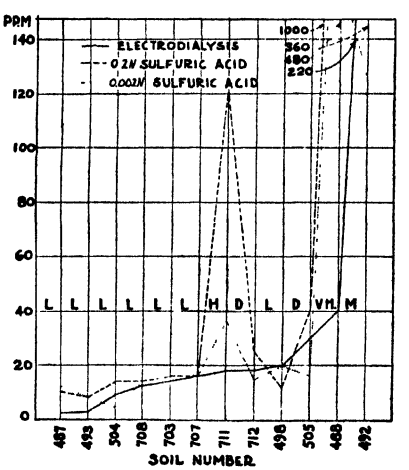

FIG. 6

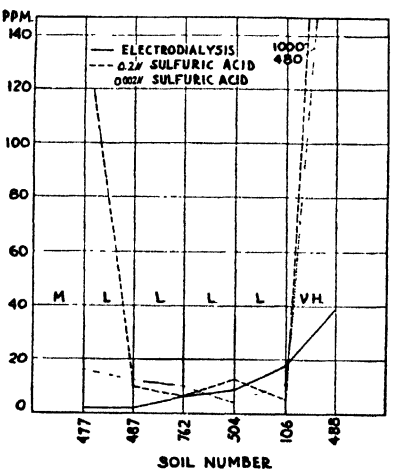

FIG. 7

FIG. 6. STUDIES ON THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, SULFURIC ACID EXTRACTION AND THE BRAY TEST, AS COMPARED WITH THE RESPONSE FROM FIELD EXPERIMENTS WITH PHOSPHATE FERTILIZERS APPLIED TO RED CLOVER

The first 9 soils beginning at the left respond to phosphorus fertilization

FIG. 7. STUDIES ON THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, SULFURIC ACID EXTRACTION, AND THE BRAY TEST, AS COMPARED WITH THE RESPONSE FROM FIELD EXPERIMENTS WITH PHOSPHATE FERTILIZERS APPLIED TO SOYBEANS

The first 5 soils beginning at the left respond to phosphorus fertilization

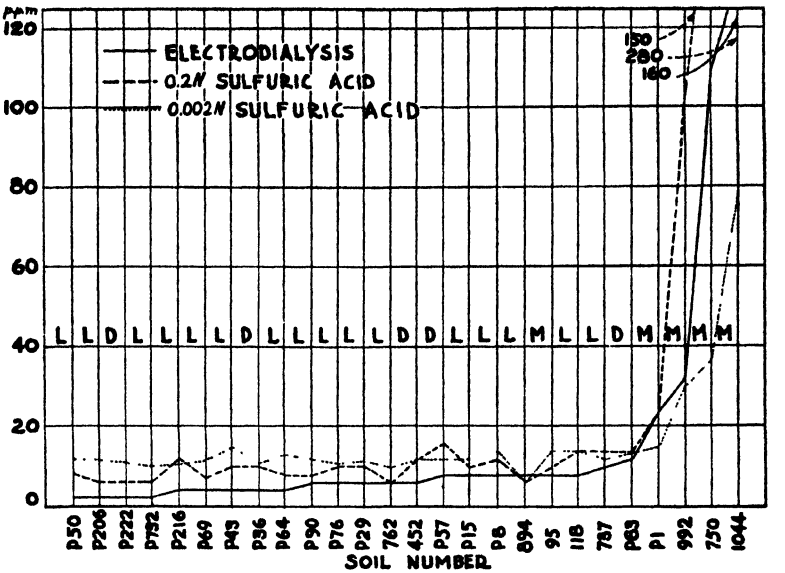

FIG. 8. STUDIES ON THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, SULFURIC ACID EXTRACTION AND THE BRAY TEST, AS COMPARED WITH THE RESPONSE FROM FIELD EXPERIMENTS WITH PHOSPHATE FERTILIZERS APPLIED TO SWEET CLOVER

All soils respond to phosphorus fertilization except the last four on the right

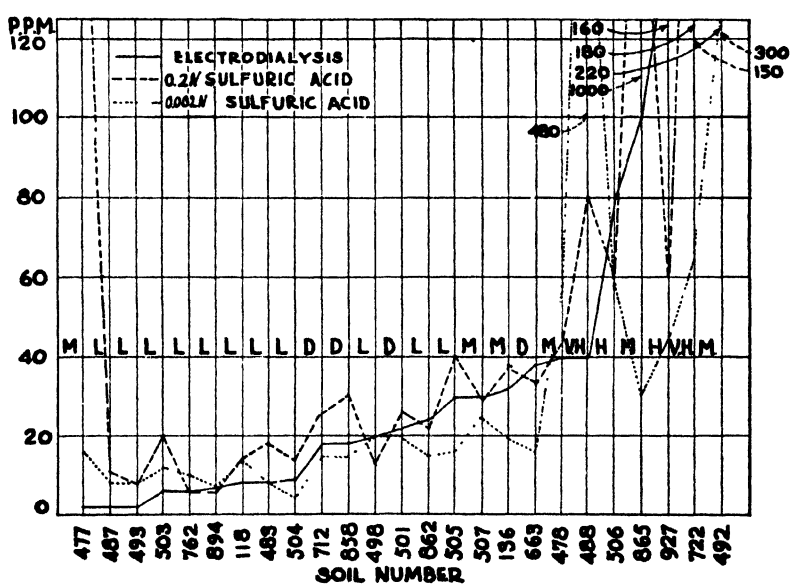

FIG. 9. STUDIES ON THE EASILY SOLUBLE PHOSPHORUS CONTENT OF SOIL AS DETERMINED BY ELECTRODIALYSIS, SULFURIC ACID EXTRACTION, AND THE BRAY TEST, AS COMPARED WITH THE RESPONSE FROM FIELD EXPERIMENTS WITH PHOSPHATE FERTILIZERS APPLIED TO WHEAT

The first 15 soils beginning at the left, except soils 858, 501, and 862, respond to phosphorus fertilization.

different methods of acid extraction is presented in figures 2 to 9 inclusive. The crops which were studied in connection with this investigation were alfalfa, corn, cotton, oats, red clover, soybeans, sweet clover, and wheat. The results obtained from some of the soils occur in three or four different charts; however because of the variation in crops grown in different rotations and in different parts of the United States, and since many samples of soil were obtained from experimental plots where only one crop such as cotton, wheat or alfalfa was being grown, it was necessary to compare the data showing the response obtained from phosphorus fertilizers applied to each crop with the amount of phosphorus removed from each soil by the four different laboratory methods which were described in a previous paragraph.

In all cases the amount of phosphorus removed by electrodialysis was used as a basis for comparison. The results obtained by the Bray test are recorded on the charts as follows: L = Low, D = Doubtful, M = Medium, H = High, VH = Very High. Those soils which respond to phosphorus fertilization are indicated in the legend of each figure.

CORRELATION STUDIES

Alfalfa. The results of soil analysis and crop response to phosphorus fertilization on alfalfa are given in figure 2. Soils which contained more than 30 p.p.m. of easily soluble phosphorus as determined by electrodialysis and 0.2 *N* sulfuric acid extraction and more than 20 p.p.m. by the Truog method did not respond to phosphorus fertilization. All of the soils which contained less than these amounts responded to phosphorus fertilization. Fifty per cent of the soils which responded to fertilization tested doubtful by the Bray method.

Corn. The results comparing the amount of easily soluble phosphorus extracted from soil and crop response from fertilizers applied to corn are shown in figure 3. In this particular experiment the agreement between the different methods was very good except that the 0.2 *N* sulfuric acid method and the Bray method extracted considerable amounts of phosphorus from soil 477. This soil contains a large amount of easily soluble iron phosphate, which apparently is not readily available for plants. All of the methods except electrodialysis gave high results on soil 711, which is a calcareous soil obtained from the Iowa Agricultural Experiment Station and probably contains considerable amounts of occluded phosphate. Soils which contained less than 20 or 30 p.p.m. of easily soluble phosphorus responded to phosphorus fertilizers applied to corn.

Because of a marked variation in climatic and soil conditions in different parts of the country, it may be difficult to determine the amount of easily soluble phosphorus above which crop response from phosphorus fertilization would not be obtained and below which response from fertilization will be profitable. In the Great Plains area very frequently drouth is a limiting factor in crop production even on soils which are deficient in phosphorus.

Also Truog (11) has pointed out that corn grown in the northern climates may need larger amounts of easily soluble phosphorus than corn grown in the Southern States because of the difference in the length of the growing season. Another factor which is also important is the amount of organic phosphorus which is liberated by biological activities in the soil.

Cotton. The three quantitative methods showed a very good correlation for easily soluble phosphorus and response from phosphorus fertilizers applied to cotton. Only one soil which was very low in easily soluble phosphorus did not respond to phosphorus fertilization. This soil was number 491 and came from College Station, Texas. On this particular soil, nitrogen is an important limiting factor in crop production.

The results obtained by the Bray test were either doubtful, medium, or high in 7 out of the 13 soils which responded to phosphorus fertilization. Also the amount of phosphorus extracted by the Truog method was rather low on the three samples of soil which did not respond to phosphorus fertilization. Soil 102 is a black waxy soil which is basic in reaction, and response from phosphorus fertilization was slight.

In comparing the results obtained from fertilizers applied to cotton and the amount of easily soluble phosphorus in the soil, it should be kept in mind that very frequently fertilizers which are applied to cotton are in all probability not applied in the ratio which will give maximum results from the standpoint of economical fertilization, especially when low and high rates of fertilization are compared. Many experiments conducted on phosphorus deficient soils indicate that the addition of a complete fertilizer will continue to give an increase in crop yield as the rate of fertilization is increased. Most experiments, however, are not designed to show which plant food is responsible for the increase in yield. It is quite possible that many soils which have been fertilized for a long time contain enough easily soluble phosphorus to produce good yields and that the effect of the addition of a complete fertilizer on the growth of plants is due to the nitrogen and potash applied. Although phosphorus is not an expensive fertilizer, there are many soils where the percentage of phosphorus in the fertilizer could be reduced considerably or eliminated completely for a while, because of the residual accumulation of phosphorus from previous treatments.

Oats. The data obtained on oats are very similar to the results presented on corn. Soil 477 and soil 711 also occurred in this comparison. The zone above which no response from phosphorus fertilization was obtained was somewhere between 20 and 30 p.p.m. of easily soluble phosphorus in the soil.

Red Clover. The results of comparing the easily soluble phosphorus content of soil and the response from phosphorus fertilizers applied to red clover give no information different from that obtained from previous charts. Soil 711 does not correlate very well with the response from phosphorus fertilization except by electrodialysis. Also the Truog method did not extract enough phosphorus from soil 505 to differentiate it from soils which do not respond to phosphorus fertilization.

Soybeans. Only six samples of soil were studied in the case of soybeans, and consequently from the few data presented accurate conclusions cannot be drawn. Soil 488, which came from the experimental plots at Lexington, Kentucky, was very high in easily soluble phosphorus except by electrodialysis, which would indicate that much of the phosphorus in this soil is occluded but can be extracted by dilute acid solutions.

Sweet Clover. All of the samples used in this study were from Oklahoma. A very good agreement occurred in all of the quantitative tests for easily soluble phosphorus, but the Bray method was doubtful or medium on six soils where marked response resulted from phosphorus fertilization. Sweet clover is a very vigorous feeder on soil phosphate, and although it gives a marked response to phosphorus fertilization it will also make a very good growth on soils which

TABLE 3

The accuracy of different methods for determining the easily soluble phosphorus in soils as compared with response from phosphorus fertilization

NUMBER	CROP	NUMBER OF SAMPLES	NUMBER OF SAMPLES IN AGREEMENT WITH RESPONSE FROM PHOSPHORUS FERTILIZATION BY.			
			Electro-dialysis	Truog method	Bray method	Extraction with 0.2 *N* sulfuric acid
1	Alfalfa	13	13	12	8	13
2	Corn	28	27	24	21	25
3	Cotton	20	18	16	10	17
4	Oats	18	18	17	13	15
5	Red Clover	12	11	10	9	11
6	Soybeans	6	6	6	5	5
7	Sweet Clover	26	26	25	20	26
8	Wheat	25	21	19	20	21
Total number of comparisons		148	140	129	106	133
Per cent agreement		...	94.5	87.1	71.6	89.8

contain rather small amounts of easily soluble phosphorus provided the soil is not too acid. From figure 8 it is quite evident that most of the soils were rather deficient in easily soluble phosphorus or else contained an abundance of this particular plant food; consequently there is a lack of definite information concerning soils which contain from 30 to 50 p.p.m. of easily soluble phosphorus.

Wheat. The correlations between the effect of phosphorus fertilizers applied to wheat and the easily soluble phosphorus content of soils as determined by the different methods are very erratic. Since winter wheat is frequently affected by climatic conditions, which may reduce the yield of this crop even though it is grown on good soils, and also since wheat needs an abundance of available nitrogen to produce a good yield, it is entirely possible that phos-

phorus has not always been the most important limiting factor in many of the experiments from which the soil samples were taken.

All Crops. The results of the correlation between the response from phosphorus fertilizers applied to eight different crops and the amount of easily soluble phosphorus extracted from the soils on which these crops were grown are presented in table 3. Since electrodialysis is not a method which is well adapted to the average laboratory, apparently the Truog method or the 0.2 *N* sulfuric acid extraction method can be recommended for noncalcareous soils. Where large amounts of easily soluble ferric or aluminum phosphate are present in the soil, the Truog method is more accurate than results from 0.2 *N* sulfuric extraction. The chief difficulty with the Truog method is that it does not remove enough phosphorus from some soils which do not respond to phosphorus fertilization so that they can be separated from those which do respond to an application of phosphate fertilizer. Recent investigation has indicated that leaching a soil with 0.1 *N* acetic acid is the most accurate method for differentiating between non calcareous or slightly calcareous soils which respond or do not respond to phosphorus fertilization. The results of these experiments will be published in another paper.

DISCUSSION

Truog (11) has suggested that most of the phosphorus in a soil was probably present either in the form of iron or calcium phosphate. Since dilute acid solutions readily dissolve calcium phosphate, only small amounts of this material should be present in those soils which respond to phosphorus fertilization. When soils which contain small amounts of easily soluble phosphorus do not respond to phosphorus fertilization they either are high in total organic matter or contain fresh organic matter which supplies enough available phosphorus as a result of the mineralization of the organic matter to meet the needs of the growing crop.

In studying the method by which plants feed, we find that the root hairs probably do not extract phosphorus from one particular point for a very long time, not to exceed 10 or 12 days in most cases; consequently when one group of root hairs perish a certain interval occurs during which no roots appear in that particular zone. In case of extraction methods or electrodialysis, all of the phosphorus which is exposed on the surface of the soil particles is attacked by the solvent. In the case of plants, only a limited number of the phosphorus particles occurring in the soil are in contact with the root hairs; consequently the distribution of the root system combined with the feeding power of plants is an important factor in determining the amount of phosphorus which different plants will extract from any particular soil, and this factor may affect in some instances the lack of correlation between field results and chemical methods for determining phosphorus availability.

In studying the results obtained by the Bray method, it was found that many of the soils which respond to phosphorus fertilization actually give

medium values and frequently give values which are classified as high from the standpoint of easily soluble phosphorus. Further investigation indicated that this difficulty was frequently caused by a lack of easily soluble iron in those particular soils. In case of soils which have considerable amounts of easily soluble iron and small amounts of easily soluble phosphorus, the presence of sufficient iron in the solution prevents the maximum development of the molybdenum blue color when the test is made. However when a soil contains small amounts of easily soluble iron and also only a small amount of easily soluble phosphorus, a blue color will appear because of the fact that there is not enough iron present in the solution to prevent the development of the blue color when the tin rod is placed in the clear soil extract. It would be better to use a weak acid solution to extract the soil and then add the ammonium molybdate reagent to the soil extract, after which the blue color could be developed by stirring the clear filtrate with a tin rod. Under such conditions the effect of the iron would be eliminated and the test would be more accurate.

Acetic acid or a buffered solution of sodium acetate is not entirely satisfactory for use in extracting the easily soluble phosphorus from soil because of the possibility of decomposition of these solutions by molds. A dilute solution of sulfuric acid can be used, or the acetic acid can be preserved by adding 5 p.p.m. of mercuric chloride, which does not interfere with the test. Truog has pointed out that very little iron is dissolved by acid solutions higher than pH 3. In this experiment, soil 477, which came from the Rhode Island Agricultural Experiment Station, was very high in easily soluble iron. It was found that very little iron was dissolved from this soil when the pH of the extracting medium was above 2.7. About ten thousand samples of soil have been studied in this laboratory, and none of them were as high in easily soluble iron as this sample. It is quite likely that any method which involves the use of an acid solution to determine the easily soluble phosphorus content of soils should be less acid than 2.7, especially when large amounts of easily soluble iron or large amounts of ferric or aluminum phosphate are present in the soil. In most of the soils studied the amount of easily soluble iron or easily soluble ferric or aluminum phosphate was very low, since relatively small amounts were dissolved by 0.2 *N* sulfuric acid, which has a pH of less than 1.

From a study of these results it appears that in most soils the amounts of easily soluble phosphorus compounds are relative and that under favorable conditions practically any of these quantitative methods will give a very good index of the availability of phosphorus to plants; although the more dilute acid solutions are preferable under certain conditions.

One of the objections to electrodialysis is that it removes all of the products of solution, which in some instances if left in the soil might depress the solubility of the phosphate ion. Leaching methods are open to the same criticism. In extraction methods the dissolved salts remain in contact with the soil and in some instances the equilibrium between the soluble phosphorus which occurs in the soil extract and that portion which is held or reprecipitated on the sur-

face of certain soil particles may not give a good index concerning the amount of phosphorus which may be dissolved by the root hairs acting upon a small percentage of the phosphate particles which occur in the soil.

SUMMARY

A study was made to compare the amount of phosphorus removed from soil by electrodialysis and dilute acid solutions with the response obtained from phosphorus fertilization. Eighty-three soils were obtained from different soil fertility experiments conducted in Oklahoma and in other parts of the United States.

A large percentage of the easily soluble phosphorus occurring in the average soil was removed in a 3-hour extraction by electrodialysis. A close correlation occurred between the amount of phosphorus removed by electrodialysis and the response of crops to phosphorus fertilization.

A graphical presentation was made of the data compared with fertilizer response from eight different crops: alfalfa, corn, cotton, oats, red clover, soybeans, sweet clover, and wheat.

In most cases soils which contain more than 30 p.p.m. of easily soluble phosphorus do not respond to phosphorus fertilization. In the case of the Truog method, very few soils produced a marked response from phosphorus fertilization when the easily soluble phosphorus was above 20 p.p.m. The Bray method for easily soluble phosphorus was not as accurate as the other quantitative methods. It was found that a lack of easily soluble iron in the soil was frequently responsible for the appearance of doubtful and medium amounts of easily soluble phosphorus by the Bray test when certain soils which give a marked response to phosphorus fertilization were analyzed.

The rate of solubility of different phosphate minerals by electrodialysis was studied. It was found that the iron and aluminum phosphates were less soluble than the calcium phosphates. Apatite, triplite, and quercyite were less soluble than chlorapatite, collinsite, collophanite, and griphite. Dufrenite, wavellite, and vivianite were the least soluble of the minerals studied.

Very little iron is dissolved from soils by solutions which are less acid than pH 2.7.

Climatic conditions are frequently interfering factors from the standpoint of determining response of soils to phosphorus fertilization as compared with the amount of easily soluble phosphorus present in the soil. Also soils which contain large amounts of organic matter may be rather low in easily soluble phosphorus, but little response is obtained from phosphorus fertilization, since enough of this element is obtained by the plant as a result of the mineralization of organic phosphorus by the bacteria and fungi in the soil.

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ESTIMATION OF PLANT AVAILABLE PHOSPHATE IN SOIL

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After some years of study and of experimenting with methods, the writer has developed procedures which appear to give as nearly as possible, with present knowledge, a fairly reliable estimate of the amount of phosphate easily available to plants in a soil. Much remains to be done before the results of examination of a soil can be interpreted with satisfactory reliability, yet the results already obtained are deemed worthy of this presentation.

The procedures described are strictly chemical laboratory methods. The information obtained by means of them is probably about as reliable as that gained by any other chemical methods and more illuminating than most others. The time and work required are very much less than are needed for the biological methods, such as those of Neubauer (6) or the various modifications of the Winogradsky (11) procedure. At the start, it is assumed that any satisfactory method must give results in general agreement with the indications given by plants growing in the same soil. It is known that what is adequate for one kind of plant may be quite insufficient for plants of another species, yet some plants must finally be relied upon to show whether a soil will produce more paying crops when phosphate fertilizers are used.

No review of the literature is given, and no discussion of published methods is offered. A considerable bibliography of the subject is given in a previous article by the writer (4). The present purpose is simply to make known, in order to leave to the judgment of others, the proposed methods. These methods were evolved from a study of those previously published, with some new invention.

Knowledge of three variables is necessary in order adequately to characterize the phosphate supplying power of a soil, the amount available to the plant at any one moment, the rate at which it will be restored to the original level when it is being withdrawn from the soil solution by any agency such as the growing plant, and the total amount which may become available during a considerable number of years. The latter may be very different from the total phosphate present in the soil. The content of some sort of acid extract has usually been relied upon to measure the amount of phosphate available at any one moment. The writer feels that most of the results thus obtained are of questionable value, likely to be overestimates. In the soil, carbonic acid is the chief agent for dissolving phosphates. Sometimes nitric acid may help. But the concentra-

tion of these acids in the soil is so small that their dissolving effect is little compared with that of the acids which have usually been employed in laboratory tests. Also, their concentration in the soil varies so greatly from time to time that it is too indefinite for a standard procedure. Since the soil water must be the chief agent for conveying soil phosphate to plants, it seems to be the logical solvent for extracting the available phosphate in a soil. Water free of CO_2 is difficult to maintain in that condition and is not like the soil solution, although it is advocated by Dirks and Scheffer (1) as the appropriate solvent for phosphate ion in nonalkaline soils. Ordinary distilled water containing some CO_2 is relatively constant in composition, is easily obtained, and is more like the soil solution than CO_2-free water; therefore it has been chosen as the most appropriate solvent for extracting soil phosphate. Better yet would be the study of actual soil solution. But it is difficult and expensive to extract it from any soil, and impossible to get from many soils. However, a 1:1 water extract is not difficult to obtain from any soil and in a majority of cases has about the same concentration of PO_4 as the actual soil solution. Table 2 shows that a 1:4 water extract will serve about as well as the 1:1 extract and, since it is easier to prepare, it is used to determine approximately the concentration of PO_4 in the soil solution.

PREPARATION OF WATER EXTRACT

The solution is prepared by shaking 50 gm. of soil with 200 cc. of ordinary distilled water in a closely stoppered bottle for an hour in an end-over-end shaker. The shaking should be very gentle. It has been found (5) that violent shaking makes a great difference in the amount of PO_4 dissolved from sandy soils. The length of time of shaking is arbitrarily set at 1 hour. Sometimes longer agitation will bring more PO_4 into solution, but 1 hour does very well for comparative purposes. After the shaking, the soil mixture is poured upon a phosphate-free filter paper in a Büchner funnel and the solution drawn through by suction. Since the first of the filtrate usually is turbid, it is returned to the filter till it comes through clear. Phosphate is determined in the filtrate by the molybdenum blue method as modified by Parker and Fudge (7). This gives a fair idea of the concentration of PO_4 in the soil solution immediately available to the plant, but it does not indicate the total easily available water-soluble PO_4 in the soil. For this purpose, a 1:100 water extract is made. This gives a solution about as dilute as is convenient for estimation of PO_4 by the molybdenum blue method, otherwise a still greater dilution would be preferable for soil having much easily soluble PO_4. The 1:100 water extract does not give the actual total water-soluble PO_4, but it does provide a figure which, for the purpose of comparison, is equivalent to the total or serves as well.

Preparation of the 1:100 water extract is similar to that of the 1:4 extra except for the method of filtration. In the latter, the soil itself is the real filtering medium, but in the 1:100 extract, there is not enough soil mass on the

filter to produce a clear filtrate by the usual method. A Büchner funnel with a 7-cm. filter such as Whatman 30 or S. and S. 589 is used with suction. To prevent creeping of the soil under the edge of the filter; suction must not be interrupted during filtration.

RECEIVER FOR CONTINUOUS FILTRATION

Accordingly a 2-bulb receiver is used. The two bulbs, one above the other, are connected by a stopcock between them, and also by a side tube provided with a 2-way stopcock connecting the upper portions of the two bulbs. The lower bulb has a stopcock for drawing off the filtrate. The Büchner funnel is connected with the upper bulb by a rubber stopper. In operation, both stopcocks between the bulbs are open at first and the filtrate collects in the lower bulb. When it begins to come through clear, the middle stopcock is closed and the other is turned to admit air to the lower bulb while the suction is held on the upper bulb so that filtration continues. The turbid liquid is drawn off from the lower bulb and returned to the filter or rejected. Now both stopcocks between the bulbs are opened again so that the filtrate collects in the lower bulb. In this manner of working, suction is continuous on the filter so that no soil can creep under the edge of the filter to make the filtrate turbid. If the soil is very coarse, it is sometimes helpful to add a little filter paper pulp to the soil suspension in order to obtain a clear filtrate.

RE-EXTRACTION OF THE SOIL

If it is desired to know something of the continued supplying power of the soil, the filter paper and soil from the first extraction are returned to the bottle with 200 cc. of water and the extraction is repeated as at first. A third extract may be prepared in the same manner. Further extracts are difficult to prepare on account of removal of electrolytes which aid filtration. Also, the added filter paper retains some PO_4 and makes the work cumbrous. The hardened filter papers from which the soil may be washed off, are not suitable because of their great tendency to retain phosphate. The three extracts obtained in this way serve very well to indicate the phosphate supplying power of the soil, both as to amount, and the rate at which it comes into solution. This method of repeated extraction is not proposed as a routine procedure. It is too laborious, but it will, in a short time, give considerable insight into the capacity of the soil to supply phosphate. Much the same information may be obtained with less labor though requiring a longer time by the percolation method.

THE PERCOLATION METHOD

In this procedure, a small portion of soil is percolated with water, or other solvent at a slow rate for 21 hours. The percolate is collected in seven portions successively so that each may be analyzed separately. After the apparatus is started, it requires very little attention till next day when the solutions are ready to be analyzed. The operation is entirely mechanical and automatic.

In order to obtain comparable results with different soils, the rate of percolation must be uniform. The same volume of reagent must pass through the same amount of soil in the same length of time. With the present apparatus, this is fairly well accomplished so long as the temperature does not vary much. Increase of temperature increases rate of flow so that hand adjustment is required to maintain constancy of flow. However this needs little attention. An apparatus intended to perform the same work was recently described (3). The present apparatus is much better than the earlier one.

THE PERCOLATION APPARATUS

On a shelf about 5 feet above the work bench stands a large bottle, fitted as a Mariotte bottle, of distilled water or other solvent. This siphons down to a 200-cc. bottle which maintains a nearly constant level supply for the siphons which, in turn supply the regulating tubes that control the rate of flow of the solvent to the percolating tubes. The regulating tubes are glass tubes about 8 mm. inside diameter, 30 cm. long, filled with clean fine acid-washed quartz sand which greatly retards the flow of the water though the tube. The tube is held at an angle of about 30° with the horizontal so that the lower end is a little below the top of the water in the constant level bottle. By suitable varying of the height of the regulating tube below the level of the water in the constant level bottle, the effective hydrostatic head on the regulating tube is changed as necessary to produce a constant rate of flow of about 40 drops a minute or 125 cc. in 3 hours. From the regulator, the water drops into an open tube which conveys it to the percolator tube.

The percolator is an ordinary filter tube such as is used for holding gooch crucibles. It is about 30 mm. inside diameter. In the bottom is a perforated porcelain plate on which rests the filtering medium. First is a layer about 4 mm. thick of paper pulp. This is held in place by a disk of brass gauze. After most of the water has been sucked out from the paper pulp by vacuum, 0.1 gm. of powdered talc suspended in 10 cc. of water is added and the water again drawn out. Then another 3-mm. layer of paper pulp is added, the water drawn out, and the whole well smoothed and packed down. A little acid-washed clean quartz sand is placed on top to aid in separating the filter from the soil in order to recover the paper pulp. Two and a half grams of the soil mixed with 5 gm. of the sand are added. On top is a wad of cotton 3 mm. thick pressed wet on top of the soil so that it will not be disturbed when the percolating water drops upon it. The layer of talc inclosed between two layers of paper pulp is the effective filtering medium, although but little of the soil passes through the upper layer of paper pulp to lodge on the talc. The whole constitutes an excellent filter which permits passage of the solvent at the desired rate and delivers an almost clear filtrate after the first 50 cc. of water have passed through. If the solvent is not pure water but contains some electrolyte to maintain flocculation of the soil, the filtrate is clear from the beginning. The percolator is closed at the top by a rubber stopper through which pass two

glass tubes. The small one, 3 mm. diameter, conducts in the solvent from the regulator tube located about 3 feet above. The other tube is about 8 mm. inside diameter and 80 cm. long, standing vertically. Its function is to allow escape of the air which is entrained along with the water, and to build up hydrostatic pressure on the percolator so that if the water does not pass through the soil and filter as fast as it comes from the regulator, the increased pressure caused by the column of water will soon be enough to maintain percolation at a constant rate. When the soil is sandy, no pressure is required. Very much clay in the soil slows filtration so that some pressure is needed to maintain the constant rate of flow.

From the percolator, the water drops into a small funnel which conducts it to the first collecting bottle. The collecting system is similar to that described in the previous paper (3) except that the bottles hold 125 cc. instead of 1,000 cc. The bottles are closed by 2-hole rubber stoppers. Through one hole passes a short open tube to permit escape of the air from the bottle. Through the other hole in the stopper passes the upright of a T tube. The horizontal arms of the "T" are connected by rubber tubing with the arm of the T tubes in the next bottle on each side so that the set of six bottles is consecutively filled by the liquid coming from the percolator. All of it drops into the first one till that is filled, then passes through the horizontal arms of the T tubes to the second bottle, and when that is filled to the third bottle, etc., till all six bottles are filled during the course of about 18 hours. Finally, the liquid from the last bottle drops into a large open jar, which is the seventh in the series. Very little of the liquid from the percolator mixes with that in the already filled collecting bottles. When the percolation is to be stopped, a pinch clamp is placed on the rubber tube which carries water from the constant level bottle to the regulating tube, thus stopping the flow but leaving the regulating tube full of water so that it will start again at once when the pinch clamp is removed.

After the extraction is finished, 100 cc. of the liquid is taken from each of the collecting bottles for determination of the phosphate by the molybdenum blue method. Another portion is used to determine the pH by indicators, and another portion is used to make an estimate of the calcium by turbidity when ammonium oxalate is added (2). Three percolators with their sets of collecting bottles are used by the writer. Analyzing the contents of the 21 bottles takes about 1½ hours. To clean out the percolators, to charge them with a new set of soils, and to set the apparatus in operation again requires 15 to 20 minutes. Thus it is possible to test a set of three soils every day in about 2 hours of the operator's time. The paper pulp used in the percolators is thrown into a large bottle of water to separate it from the soil and sand so that it may be recovered for further use.

In principle, this process is similar to the successive extraction method of von Wrangell (12) or the collodion bag diffusate method described by Pierre and Parker (8). It has the advantage over these of requiring no elaborate or expensive apparatus and much less of the operator's time and labor. In this

fairly simple manner, it is possible to measure the amount of easily soluble phosphate in the soil, the time rate at which it will be supplied by the soil, the level at which it will be supplied after the first easily soluble portion has been removed, and the pH of the solutions which indicate the buffer power of the soil, and give some idea of the amount of easily soluble calcium in the soil. The calcium thus dissolved seems to control somewhat the solubility of the phosphate (see table 4).

TOTAL EASILY SOLUBLE PHOSPHATE IN THE SOIL

In most soils, there is some phosphate not easily extracted by water but readily dissolved by dilute acids. Various workers have used a number of different acids of different concentrations. The writer has used 0.05 N HCl and 0.05 *N* H_2SO_4. The latter seems to have greater solvent power, therefore it has been preferred. For the extraction, 1 gm. of soil is placed in a Jena sintered glass filtering funnel, porosity 3, with 30 mm. filtering plate. A gooch filter with asbestos might be used if packed tight enough to make percolation slow. A 500-cc. flask with narrow neck is filled with 0.5 *N* H_2SO_4 and inverted with the mouth just above the soil and held by a clamp so that the liquid may run into the filter as fast as it percolates through. Several hours are required for the 500 cc. to pass through the soil and filter. After this, the collecting bottle is changed, the supply flask refilled, and another 500 cc. of extract is obtained. Next time, only 200 cc. of acid is placed in the supply flask and the percolate is received in another bottle. When the extraction is finished, the PO_4 in the three portions of extract is determined by the color method, as before stated. Usually, most of the phosphate is contained in the first 500 cc. of extract, with a little in the second and almost none in the third, showing that the soil has been exhausted of easily soluble PO_4. The extract is collected in three separate portions which are analyzed separately in order to determine something in regard to the ease of solubility and whether the extraction has been complete. One objection to this procedure is the long time required, which may be 2 or 3 days, for a single sample of soil. But in most cases, this is not important, since very little of the operator's time is needed. It is possible to draw the acid through the filter very much faster by the aid of suction, but a few experiments showed that the extraction was not so complete as when it proceeded by gravity over a longer time. Considerable time seems to be an essential feature of the process. Somewhat similar figures are obtained by the Truog method (9). But since the latter is an equilibrium process, it is not to be expected that the results obtained by the two methods should be alike. Probably the figures obtained by the Truog method will serve almost equally well to characterize the soil in most cases, with much less time and perhaps less labor. To make the matter clearer to those not familiar with it, a few details of the Truog method are given here.

TRUOG METHOD

Two grams of 20-mesh soil are shaken with 400 cc. of 0.002 *N* H_2SO_4 containing 0.3 per cent of ammonium sulfate for half an hour. The mixture is filtered and PO_4 determined in the clear filtrate by the molybdenum blue method as described by Truog and Meyer (10).

By the use of the methods described in the foregoing, non-alkaline soils are examined as to their content of readily available phosphate. The following outline of procedure is suggested as the shortest possible which will give the needed information. Probably about the same procedure may be used with peat soils. Alkaline or calcareous soils must be tested by different reagents and perhaps by different procedures.

ESTIMATING THE AMOUNT OF PLANT AVAILABLE PHOSPHATE IN A SOIL

Test for carbonate by adding HCl, and determine pH by a suitable indicator, cresol red or phenolphthalein. If the soil effervesces or has a pH over 8, it is to be considered as alkaline and must be tested for PO_4 by other special methods. If the soil is not alkaline make the Truog test. If the PO_4 is very high or very low, no other test is needed to characterize the soil. If the Truog test indicates a medium amount of PO_4, other tests are needed.

Make water extracts 1/1 or 1/4 and 1/100. If the concentration of PO_4 in both is above 0.2 p.p.m. and above 500 in the Truog test, no other test is needed, but if the Truog test is 300 or less, it may be well to make the test by percolation with water, and with 0.05 *N* H_2SO_4.

INTERPRETATION OF THE RESULTS

In most sandy soils, and in some loams and clays, the concentration of PO_4 is higher in the 1/1 or 1/4 than in the 1/100 water extract indicating some relatively easily water-soluble PO_4. Successive 1/100 water extracts or percolation extracts of such a soil are likely to show rapidly decreasing concentrations of PO_4 so that the soil will be soon depleted of available PO_4 by heavy cropping, and may, within a few years, need phosphate fertilizer. But if there is little difference between the concentration of PO_4 in the 1/4 and the 1/100 water extracts, the soil contains very little easily water-soluble PO_4. If the concentration is below 0.2 p.p.m., it is probable that it will pay to use a phosphate fertilizer for such crops as potatoes or tomatoes which need plenty of available phosphate. If it is below 0.1 p.p.m. and the Truog test gives less than 300 p.p.m. PO_4, the soil is rated very poor in available phosphate. Probably the soil will be unable to supply available phosphate fast enough to grow good crops of barley or alfalfa. If it is over 0.2 p.p.m. in the 1/100 water extract and over 600 by the Truog test, the soil is not likely to need phosphate for some years. A fine textured soil is likely to have a more lasting supply of phosphate than a coarse soil which gives the same amount in any of the tests.

Soils which have a high level at first, such as 0.5 to 1+ p.p.m. in 1/100 water

TABLE 1

PO_4 extracted by various methods

SOIL NUMBER	TEXTURE	$CaCO_3$	pH	FUSION	0.2 *N* HNO_3	PERCOLATE WITH 0.05 *N* H_2SO_4	TRUOG EQUILIBRIUM 0.002 *N* H_2SO_4	WATER		
								Percolation	Equilibrium 1:1 or 1:4	Equilibrium 1.100
		per cent		*p.p.m.**	*p.p.m.**	*p.p.m.**	*p.p.m.**	*p.p.m.**	*p.p.m.†*	*p.p.m.†*
1c	Silty clay loam	0	6 7	2,000	400	1,104	620	60	0.20	0.18
30	Sandy loam	0	6.9	1,600	1,120	1,250	440	68	5.00	0.40
35	Sandy loam	0	5 4	2,400	80	527	20	6	0 04	0.03
36	Sandy loam	0	6.8	2,500	220	802	330	42	0 12	0 14
37	Sandy loam	1 0	8.2	2,400	250	870	400	39	0.58	0.18
38	Clay loam	0	7.2	2,500	80	876	248	60	0 18	0 21
40	Loam	0	6.3	3,300	1,080	2,042	800	340	5.00	0.88
53	Fine sand	0	7.1	800	500	680	88	37	1 32	0 27
59	Clay loam	0	6.0	2,000	2	134	4	0	0	0
64	Loam	0	6 9	1,700	270	979	160	20	0 10	0 08
65	Silty clay loam	0	7.1	2,800	.....	1,529	800	154	2 00	0 72
68	Sandy loam	0	7 5	1,400	.....	1,460	200	35	0 24	0 10
78	Sandy loam	0	4 8	450	.....	118	20	13	0 20	0 06
80	Loam	0 4	8 3	1,150	500	722	260	20	0 16	0 06
A	Peat	.....	..	....	.....	1,041	100	44	0 04	0 04
B	Peat	.....	...	.....	.....	662	88	66	0 26	0 28
C	Peat, same as B plus superphosphate	.....	...	.....	.....	1,165	288	200	0.48	0 68

Rich in available phosphate: 1c, 30, 40, 65.

Medium in available phosphate: 36, 37, 38, 53, 68.

Poor in available phosphate: 35, 59, 64, 78, 80.

36, 37, 38 are too low in water soluble.

53 has too little acid soluble, though it is high in water soluble.

80 has plenty of PO_4, but is too alkaline.

* In soil. † In solution.

Comments: Some figures indicating the general character of the soils and their response to the phosphate tests are presented. In some cases, the total PO_4 has no relation to availability, as in 35, 36, 37, 59, 64, and 80. The PO_4 dissolved by the comparatively strong acid, 0.2 *N* HNO_3, in some cases gives a correct estimate of the availability. But in the cases of 38, 53, 64, and 80, its indication is misleading. Of these, 38 is the best in respect to ability to supply growing plants with adequate PO_4. In the greenhouse, 38 has demonstrated its superiority in growing nine consecutive crops of barley and tomatoes. Soil 64 contains more easily acid-soluble PO_4 than 38, but it cannot give a high enough concentration in the water extracts. Soil 53 has relatively high easily water-soluble PO_4 but the total easily available PO_4 (Truog) is much too low. It is quickly exhausted by cropping. Soil 80 is too alkaline to permit easy solution of its good supply of easily acid-soluble PO_4.

Both dilute acid-soluble (Truog), and water-soluble phosphate are so high in soils 1c, 30, 40, and 65 that these are at once classed as rich soils, whereas by the same tests, 35, 59, and 78 are very poor. The figures indicate that 37 is better than 38, yet all pot culture work shows that 38 is one of the best in the whole lot, whereas 37 is in the medium to poor class. This shows that the chemical tests are not always capable of giving the correct rating to a soil. In this case, however, 37 is so alkaline, pH 8.2, that its phosphate is relatively poorly available, the same as in 80.

extract, but which level rapidly falls off with successive extracts, are likely to produce large crops at first, if other nutrients are adequate, but soon fall off in productivity.

Total PO_4 or strong acid-soluble PO_4 is frequently no guide to the amount of available phosphate in the soil, unless, when very low, it shows an absolute deficiency. Dilute acid-soluble, Truog or 0.05 *N* H_2SO_4 percolate-soluble phosphate, probably measures the total easily available PO_4, or all that plants are likely to be able to obtain in many years.

To obtain a better idea of the power of the soil to maintain an adequate supply of phosphate for heavy cropping, the test by percolating with water is

TABLE 2

PO_4 extracted from soils by water in various ratios of soil to water

SOIL		PO_4 IN THE SOLUTION AT RATIOS OF SOIL TO WATER				
		1/1	1/5	1/20	1/50	1/100
		p.p.m.	*p.p.m*	*p.p.m.*	*p.p.m*	*p.p.m.*
1c	*A. Loams*	0 20	0 36	0 22	0 22	0 18
38		0 18	0 20	0.22	0 22	...
64		0 10	0 08	0 08	0 08	0 10
68		0 24	0 20	0 10	0 10	....
80		0 16	0 16	0 12	0 10	0 06
65		2 00	2 40	1 74	1 14	0 72
30	*B. Sandy soils*	8 00	2 90	1 16	0 58	0 30
37		0 58	0 50	0 40	0 22	...
53		1 70	1 00	0 50	0 31	....
35		0 04	0 03	0 04	0 05	0 03
68		0 24	0 20	0 10	0 10	0 07

A soils have little easily water-soluble PO_4.

B soils have considerable easily water-soluble PO_4.

Usually this easily water-soluble PO_4 is rapidly diminished by intensive cropping, while the less easily soluble PO_4 in the *A* soils may supply PO_4 rapidly enough to produce good crops many years if it is high enough at the start.

This table is presented in order to show that in many cases, the ratio of soil to water makes little difference in the concentration of PO_4 in the extract.

most illuminating. Three successive extracts at 1/100 ratio give the same kind of information in much shorter time but requiring much more labor. A graph made by plotting the results of the percolation test shows at a glance the character of the soil with regard to phosphate.

The buffer power of the soil and the rate at which it reacts with a neutralizing agent are very important in laboratory tests where time is an important factor. In the field, the time rate may be unimportant, but in the laboratory if too little time is allowed for the test, the buffer power may be much underestimated.

Tables 1 to 4 with appended notes are self-explanatory and are included to give the reader an idea of the magnitudes involved in this sort of soil tests

PO_4 extracted from soils by percolation with water compared with PO_4 in successive equilibrium extracts

p.p.m. PO_4 in soil

SOIL	METHOD*	EXTRACT NUMBERS							TOTAL
		1	2	3	4	5	6	7	
1c	P.	0.16	0 09	0 06	0.06	0 06	0.06	0.06	0.55
	E.	0.20	0.15	0.22	....	....	....	....	0.57
30	P.	0 31	0 10	0.09	0 08	0.07	0.07	0.06	0.78
	E.	0.54	0.34	0.08	....	....	....	....	0.96
35	P.	0.01	0 02	0 02	0.02	0 01	0.01	0.01	0.10
	E.	0 06	0 02	0.02	....	....	....	....	0.10
36	P.	0.10	0 08	0 06	0 05	0 04	0.04	0.03	0 40
	E.	0.14	0 12	0 10	....	..	....	....	0 36
37	P.	0.13	0 06	0.05	0.05	0.04	0.04	0 03	0 40
	E.	0 17	0 10	0 03	....	....	....	....	0.30
38	P.	0 15	0 13	0 08	0 07	0 06	0 05	0 05	0 59
	E.	0.20	0.08	0 08	....	....	....	....	0 36
40	P.	1.25	0.50	0 33	0.20	0 15	0 11	0 22	2.62
	E.	0 88	1 00	1 00	....	....	....	....	2 88
53	P.	0.15	0 06	0 05	0.04	0 03	0 03	0 02	0 38
	E.	0 27	0 07	0 06	.. .	....	....	....	0 40
64	P.	0 07	0 06	0 03	0 03	0 02	0 02	0 01	0 24
	E.	0 08	0 04	0 06	....	.. .	....	....	0.18
65	P.	0 50	0 28	0 28	0 15	0 11	0 11	0 10	1.53
	E.	0 32	0 44	1 00	...	....	....	....	1 76
68	P.	0 07	0 06	0 06	0 05	0 05	0 04	0 03	0 36
	E.	0 14	0 08	0 05	....	...	....	.. .	0 27
78	P.	0 03	0 03	0 03	0 02	0 02	0 01	0 01	0 15
	E.	0 06	0 04	0 04	....	....	....	....	0 14
80	P.	0 01	0 04	0 05	0 05	0 05	0.06	0 07	0 33
	E.	0 06	0 04	0 02	....	.. .	....	....	0.12

* P. = percolation; E. = equilibrium.

Comments: Table 3 permits a comparison of the amounts of PO_4 extracted by continuous percolation and by successive equilibrium extracts. It also gives an idea of the rate at which the different soils give their PO_4 up to a water extract. The equilibrium extracts have higher concentration than the percolation extracts because all the PO_4 that can dissolve in the water is entirely removed by filtration at each extraction, which is quite different from the percolation process in which there is no such complete separation between successive percolates. However, it is evident that the quantities found by the two procedures run parallel, thus giving a similar character to the soil.

The figures show how rapidly the easily soluble PO_4 is removed from rich soil such as 40 and 65, how the high buffer alkalinity of 80 retards solution of PO_4, how the poor character of 35 and 78 is indicated by the low concentrations in the extracts, and why soil 38 is so much better than 64, although the latter has more PO_4 easily soluble in dilute acid, 0.2 *N* HNO_3 (table 1). Soil 38 has a much higher rate of solution in water, and a higher total PO_4 which enables it to maintain for a long time a high enough concentration in the soil solution to produce good crops.

TABLE 4

Tentative standards for availability of soil phosphate as estimated by various methods

KIND OF SOIL	PO_4 IN SOIL		PO_4 IN SOLUTION—EQUILIBRIUM WATER EXTRACTS	
	Truog acid soluble	Water by percolation	1:1 or 1 4	1 100
	p p m	*p p m*	*p p m*	*p p m*
Rich silty clay loam...	Over 500	Over 60	Over 0.25	Over 0 20
Medium loam.....	200–500	30–60	0 15–0 20	0 15–0 20
Poor loam	Below 200	Below 30	Below 0 10	Below 0 10
Rich sandy loam .	Over 600	Over 80	Over 5 00	Over 0 40
Medium sandy loam .	300–600	35–70	0 25–1 00	Over 0 20
Poor sandy loam..	Below 300	Below 20	Below 0 25	Below 0 15

Analysis of more soils of well-known field behavior in respect to phosphate supplying power will be necessary to establish the limits more definitely.

Comments: Table 4 sets forth tentative standards to be used in interpreting an analysis of a soil by the proposed methods. Soils in the class "rich" are not likely to be able to produce much better crops from applications of phosphate fertilizers. The "poor" soils are likely to need phosphate in order to produce good crops. The medium soils may give good yields of some crops for several years without receiving any phosphate fertilizer, but for good yields of plants requiring much PO_4, it may pay to use fertilizer at first. Much more work must be done with soils of known field behavior before it will be possible to interpret the results of an analysis of an unknown soil with any great degree of precision.

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COMPARISONS IN THE DISTRIBUTION OF NEMATODE GALLS ON THE ROOTS OF PINEAPPLE VARIETIES ATTACKED BY THE NEMATODE HETERODERA RADICICOLA (GREEF) MÜLLER[1]

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Pineapple plants are quite generally infected by the root knot nematode in the Hawaiian Islands except for certain areas to which it has not yet been distributed. An analysis of the damage to the roots of several varieties of young plants has been made recently by Collins and Hagan (2). Some additional data, not given in their report but obtained from the same experiments, may have considerable economic and theoretical significance quite apart from a comparative study of the injury suffered directly by the crop attacked. This information consists of comparisons between varieties of pineapples in the number of galls produced, their distribution on the roots, their relation to reduction in root length, and their influence upon the future nematode population of the soil. The following information is briefly presented in order to make these comparisons: Differences between varieties in the number of non-terminal galls per plant; Differences between varieties in the number of terminal galls per plant; Differences between varieties in the total number of galls per plant; Differences between varieties in the number of galls per root; Differences between varieties in the number of galls per foot of root length.

Nematode infection also is present in the fine fibrous roots, but no tabulations of data on this subject have been included. Some pineapple varieties have few such roots whereas others have dense masses of them. Observations of this condition have shown no reliable method of evaluating infection in the fibrous roots which could be used in comparative studies. The galls are very small. Many are lost in freeing the roots from soil, the greatest absolute loss occurring on plants with the largest numbers of such roots. Likewise, it has been impossible to make any estimate of the abundance of nematodes within the older parts of the roots, where they form no galls. The labor necessary, the time consumed, and the resultant damage to the plant through drying and breaking of fibrous roots make these analyses impracticable.

This paper includes comparative studies on the following varieties of pineapples: Cayenne, Hilo, Lot 520 (F_1 hybrid of Cayenne x Wild Brazil), Natal,

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Pernambuco, Ruby, Taboga, and Wild Kailua. Only the first two are grown commercially in the Hawaiian Islands, but all of them are used for investigational work at the Pineapple Experiment Station. Sixteen plants of each variety were grown, except that there were 12 Cayenne and 32 Hilo plants in the test. Each container, of 3 gallons soil capacity, held two plants. A uniform, fairly heavy inoculation of 50 nematode egg masses was placed in each container shortly after root growth started. The plants were permitted to grow approximately 8 months before being removed from the soil for these observations. Further information regarding the routine of the experiment may be obtained from the more detailed report by Collins and Hagan (2).

The galls found in this analysis have been divided into two groups according to their location on the roots. Those situated apically are classed as terminal galls, whereas others, scattered along the roots and not at the root tips, are called "non-terminal galls." In the tables presented, the figures given represent arithmetical differences between varieties in the number of galls possessed by them in the comparisons that are made. The probable errors of the differences also have been entered that the reader may judge how significant the figures may be or how closely such differences approach significance. Regardless of significance, arrows point to the variety with the greater number of galls in each comparison, and the letter *s* has been added to significant differences in order that they may be more easily detected.[2]

DISTRIBUTION OF GALLS

Differences between varieties in the number of non-terminal galls per plant are shown in table 1. Ruby has fewer than Cayenne, Hilo, Lot 520, or Natal; Taboga fewer than Hilo, Lot 520, or Natal; Wild Kailua has a smaller number than Hilo or Natal. The remaining differences are not statistically significant, yet a definite trend manifested in two of the varieties, Natal and Ruby, should not pass unnoticed, as it is entirely possible that these differences might assume significance with larger populations of plants. In all comparisons, the arrows are directed away from Ruby, indicating that this variety has the fewest non-terminal galls. Natal, on the contrary, probably has the greatest number, as all of the arrows point toward this variety.

Table 2 gives the differences between varieties in the number of terminal galls per plant. There are 22 significant differences in the 28 comparisons. Taboga has the fewest galls. Cayenne and Hilo have an equal number, which

[2] Since this consideration of the purely nematological phases of pineapple infection originates from data gathered in tests already reported by Collins and Hagan (2) on the nematology-genetics aspects of the work, the writer is indebted to the same sources for help in recording the original data. Further, through the courtesy of the *Journal of Heredity*, some information appearing in the afore-mentioned article, important here for the purposes of reference or comparison, has freely been drawn upon, especially in the preparation of table 6. Some slight discrepancies appear between these figures and those given in the original paper, since the present figures have been carried to one more decimal point.

greatly exceeds the number of terminal galls possessed by any of the other varieties. Since none of the varieties is immune to attack, we would expect to find that those with the greatest number of main roots would also have more terminal galls per plant; and, upon inspection, such is found to be the case.

A comparison of the figures in table 1 with those in table 2 shows that in every instance, where an actual difference exists between varieties in the number of

TABLE 1

Differences between varieties in number of non-terminal galls per plant

Arrow indicates variety with greater number; *s* indicates a significant comparison.

VARIETY	CAYENNE	HILO	LOT 520	NATAL	PERNAMBUCO	RUBY	TABOGA
Hilo	1 78 ±2 40 ←						
Lot 520	0 41 ±2 41 ↑	2 19 ±1 90 ↑					
Natal	2 59 ±2 49 ←	0 81 ±2 00 ←	3 00 ±2 02 ←				
Pernambuco	2 78 ±3 24 ↑	4 56 ±2 87 ↑	2 37 ±2 89 ↑	5 37 ±2 95 ↑			
Ruby	10 16 ±2 03 *s* ↑	11 94 ±1 38 *s* ↑	9 75 ±1 41 *s* ↑	12 75 ±1 54 *s* ↑	7 38 ±2 58 ↑		
Taboga	7 10 ±2 16 ↑	8 88 ±1 57 *s* ↑	6 69 ±1 60 *s* ↑	9 69 ±1 71 *s* ↑	4 32 ±2 68 ↑	3 06 ±0 92 ←	
Wild Kailua	6 73 ±2 20 ↑	8 51 ±1 62 *s* ↑	6 32 ±1 64 ↑	9 32 ±1 75 *s* ↑	3 95 ±2 71 ↑	3 43 ±0 99 ←	0 37 ±1 24 ←

non-terminal galls, there is a correspondingly significant variation in the number of terminal galls, except in the case of the Lot 520-Ruby comparison. It might be well, also, to point out at this time that ratios of terminal galls to total galls present on the roots of each variety, expressed in percentages of total galls, are as follows: Ruby, 77 per cent; Cayenne, 71 per cent; Hilo, 68 per cent; Pernambuco, 67 per cent; Wild Kailua, 59 per cent; Taboga, 57 per cent; Natal, 55 per cent; Lot 520, 54 per cent. From these figures it will be

seen that table 2 takes into consideration the majority of the galls in the roots of the plants.

Table 3 gives the differences between varieties in the total number of galls per plant. The 28 comparisons show significant differences in 20 cases and 19 of these are duplicates of differences found in table 2. Not only are the differences significant in both cases, but their arrows agree in direction as well.

TABLE 2

Differences between varieties in number of terminal galls per plant

Arrow indicates variety with greater number; *s* indicates a significant comparison.

VARIETY	CAVENNE	HILO	LOT 520	NATAL	PERNAMBUCO	RUBY	TABOGA
Hilo	2 13 ±2.70 ↑						
Lot 520	20 25 ±2.26 *s* ↑	18.12 ±1 59 *s* ↑					
Natal	15 28 ±2.44 *s* ↑	13 15 ±1 83 *s* ↑	4 97 ±1 07 *s* ←				
Pernambuco	12 50 ±2.53 *s* ↑	10 37 ±1 96 *s* ↑	7 75 ±1 30 *s* ←	2 78 ±1 58 ←			
Ruby	21 69 ±2 30 *s* ↑	19 56 ±1 65 *s* ↑	1 44 ±0 72 ↑	6 41 ±1.16 *s* ↑	9.19 ±1.36 *s* ↑		
Taboga	26 75 ±2 32 *s* ↑	24 62 ±1.67 *s* ↑	6.50 ±0.78 *s* ↑	11 47 ±1 20 *s* ↑	14.25 ±1.39 *s* ↑	5 06 ±0 90 *s* ↑	
Wild Kailua	25.68 ±3.02 *s* ↑	23.55 ±2.56 *s* ↑	5 43 ±2 09 ↑	10 40 ±2.28 *s* ↑	13.18 ±2 38 *s* ↑	3 99 ±2.13 ↑	1 07 ±2 15 ←

One significant difference given here that does not appear significant in table 2, is the Lot 520-Ruby comparison. This comparison owes its significance in table 3 to the great difference between the two varieties in number of non-terminal galls, table 1. On the other hand, table 3 lacks the statistically significant differences which exist in table 2 for Lot 520-Natal, Lot 520-Pernambuco, and Ruby-Taboga comparisons. Even when significant differences are lacking in tables 2 and 3, it will be observed that the arrows agree in direction, except for the Natal-Pernambuco comparisons.

In table 4 are found the differences between varieties in the number of galls

per root. Cayenne, Hilo, and Lot 520, have almost equal infections in this classification. Natal has more galls per root than Hilo, Lot 520, Ruby, or Wild Kailua. Ruby has significantly fewer than Lot 520, Natal, Pernambuco, or Taboga, and in every comparison the arrow points away from this variety.

One sees, in table 5, only four significant differences between varieties in the number of galls per unit of root length. Cayenne has more galls than Lot 520.

TABLE 3

Differences between varieties in total number of galls per plant

Arrow indicates variety with greater number; *s* indicates a significant comparison.

VARIETY	CAYENNE	HILO	LOT 520	NATAL	PERNAMBUCO	RUBY	TABOGA
Hilo	0 35 ±3 67 ↑						
Lot 520	20 54 ±3 61 *s* ↑	20 19 ±3 44 *s* ↑					
Natal	13 54 ±3 36 *s* ↑	13 19 ±3 16 *s* ↑	7 00 ±3 10 ←				
Pernambuco	14 72 ±3 51 *s* ↑	14 37 ±3 33 *s* ↑	5 82 ±3 27 ←	1 18 ±2 98 ↑			
Ruby	31 98 ±2 84 *s* ↑	31 63 ±2 61 *s* ↑	11 44 ±2 53 *s* ↑	18 44 ±2 15 *s* ↑	17 26 ±2 38 *s* ↑		
Taboga	33 75 ±2 97 *s* ↑	33 50 ±2 75 *s* ↑	13 31 ±2 68 *s* ↑	20 31 ±2 32 *s* ↑	19 13 ±2 54 *s* ↑	1 87 ±1 48 ↑	
Wild Kailua	27 79 ±3 22 *s* ↑	27 44 ±3 02 *s* ↑	7 25 ±2 96 ↑	14 25 ±2 64 *s* ↑	13 07 ±2 83 *s* ↑	4 19 ±1 93 ↑	6 06 ±2 13 ←

Taboga has more than Lot 520, Natal, and Wild Kailua. Although not statistically significant, is it interesting to note that all arrows, indicating arithmetical differences, point toward Cayenne, and all are directed away from Wild Kailua.

DISCUSSION AND APPLICATION OF DATA

Uniformity of infection

Table 5 is especially interesting and significant in this paper since it reveals so few statistically important differences between varieties in the number of

galls per unit of root length. Regardless of size, vigor, or kind of plant, the number and length of roots, or other varietal characteristics of growth, we find that nematode attack is remarkably uniform in all varieties, when the relative root lengths are taken into consideration together with the number of galls formed on them. Even two of the four cases of significant differences found in the table are only barely significant. That is, the difference between Cayenne

TABLE 4

Differences between varieties in number of galls per root

Arrow indicates variety with greater number; *s* indicates a significant comparison.

VARIETY	CAYENNE	HILO	LOT 520	NATAL	PERNAMBUCO	RUBY	TABOGA
Hilo	0.080 ±0 075 ↑						
Lot 520	0.013 ±0 085 ←	0 093 ±0.054 ←					
Natal	0.335 ±0.086 ←	0.415 ±0 055 *s* ←	0 322 ±0 068 *s* ←				
Pernambuco	0.145 ±0.091 ←	0 225 ±0 063 ←	0 132 ±0 074 ←	0 190 ±0.075 ↑			
Ruby	0 214 ±0 076 ↑	0.134 ±0 040 ↑	0 227 ±0 056 *s* ↑	0.549 ±0.057 *s* ↑	0 359 ±0.064 *s* ↑		
Taboga	0.133 ±0.102 ←	0.213 ±0.078 ←	0.120 ±0.088 ←	0 202 ±0.088 ↑	0 012 ±0.093 ↑	0.347 ±0 080 *s* ←	
Wild Kailua	0.052 ±0.087 ↑	0 028 ±0 058 ←	0.065 ±0.070 ↑	0.387 ±0.071 *s* ↑	0.197 ±0.077 ↑	0.162 0.060 ←	0.185 ±0 090 ←

and Lot 520 has a probable error which bears a ratio to the difference (D/E) of 4.06; the ratio between difference and probable error of the Taboga-Wild Kailua comparison is almost equally small (D/E equals 4.18). The Lot 520-Taboga value of D/E is 5.38 and Natal-Taboga D/E is 5.23.

The significant differences of Taboga when compared with Lot 520, Natal, and Wild Kailua, may express a true varietal difference between this variety and the others in the matter of relative susceptibility to attack, but with the

small number of plants used in the test the relatively greater apparent susceptibility of Taboga cannot be established as a fact. However, it may be pointed out that, except for the Cayenne-Taboga comparison, all arrows point toward this variety in table 5. Similarly, two other varieties, Cayenne and Wild Kailua, have already been mentioned because their differences, when compared with the others, seem to be constantly in favor of one and unfavorable to

TABLE 5

Differences between varieties in numbers of galls per foot of root length

Arrow indicates variety with greater number; *s* indicates a significant comparison.

VARIETY	CAYENNE	HILO	LOT 520	NATAL	PERNAMBUCO	RUBY	TABOGA
Hilo	1 07 ±0 52 ↑						
Lot 520	1.87 ±0 46 *s* ↑	0 80 ±0 32 ↑					
Natal	1 77 ±0 45 ↑	0 70 ±0.31 ↑	0 10 ±0 18 ←				
Pernambuco	1 38 ±0 47 ↑	0 31 ±0 34 ↑	0 49 ±0 24 ←	0 39 ±0 21 ←			
Ruby	1.15 ±0 46 ↑	0 08 ±0 33 ↑	0.72 ±0 21 ←	0.62 ±0 19 ←	0 23 ±0 24 ←		
Taboga	0 20 ±0 52 ↑	0 87 ±0.40 ←	1 67 ±0 31 *s* ←	1 57 ±0 30 *s* ←	1 18 ±0 33 ←	0 95 ±0 32 ←	
Wild Kailua	2.04 ±0 55 ↑	0 97 ±0 44 ↑	0 17 ±0 37 ↑	0.27 ±0 35 ↑	0 66 ±0 38 ↑	0 89 ±0.37 ↑	1.84 ±0.44 *s* ↑

the other in the number of galls per foot of root length. Aside from these possible exceptions, we must conclude that table 5, in general, shows equal infection of the varieties of pineapple plants tested, when varietal differences in root length are considered.

The variation in the number of terminal and non-terminal galls possessed by the different varieties of pineapples must be accounted for in other ways than by assuming a differential in susceptibility. The average number of non-

TABLE 6

Pineapple varieties listed to show increasing values in average numbers of non-terminal galls, terminal galls and roots, and average length of roots

A correlation is evident in the listings in columns 1 and 2 and also in columns 3 and 4.

NON-TERMINAL GALLS		ROOT LENGTHS IN FEET		TERMINAL GALLS		ROOT NUMBER	
Variety	Average number	Variety	Average length	Variety	Average number	Variety	Average number
Ruby	4 25 ±0 37	Taboga	4.95 ±0 39	Taboga	10.00 ±0 67	Taboga	10.81 ±0.53
Taboga	7.31 ±0.84	Ruby	6.96 ±0 32	Wild Kailua	11 07 ±2 05	Ruby	16 93 ±0.57
Wild Kailua	7.68 ±0.92	Wild Kailua	12 20 ±0.80	Ruby	15 06 ±0.60	Wild Kailua	17.62 ±1.22
Pernambuco	11 63 ±2.55	Pernambuco	14 71 ±0 78	Lot 520	16 50 ±0 42	Natal	25.00 ±0.22
Lot 520	14 00 ±1.36	Lot 520	14 84 ±0.94	Natal	21 47 ±1.00	Lot 520	26.90 ±1.89
Cayenne	14.41 ±2 00	Cayenne	15 51 ±1.29	Pernambuco	24 25 ±1.22	Pernambuco	28.38 ±1.25
Hilo	16.19 ±1.33	Natal	17.42 ±0 77	Hilo	34 62 ±1 54	Cayenne	38.66 ±1.76
Natal	17 00 ±1.49	Hilo	20 21 ±0 82	Cayenne	36.75 ±2.23	Hilo	42.44 ±1.25

terminal galls in each variety seems to vary with the average length of roots. Varieties with shorter root systems have fewer of them while those with longer root systems have more. Terminal galls, on the other hand, vary directly with the average number of main roots grown in each variety of pineapple. Only exceptionally will more than one terminal gall occur at the tip of a root, although this condition can be found when a lateral root pushes out beside the first terminal gall and, in turn, is attacked by nematodes. Some root tips, naturally, escape infection up to the time the tests are closed, so a perfect correlation between number of roots and number of terminal galls is not expected. It follows, however, that varieties with a greater number of roots may be found to possess a proportionally higher number of terminal galls. It is physically impossible for a variety with few roots to have as many terminal galls as one with many roots.

Comparisons of the two classes of galls with average root length and average number of roots may be made by glancing at table 6. As one reads down each column, the values increase in amount until the last plant mentioned will be observed to have the highest value in that column. Comparing the order in which the plants are placed under "Non-terminal Galls" with the list in column 2, one can readily see that the arrangement of the plants in the two columns is almost identical. Similarly, columns 3 and 4 may be compared with like results. Had these two sets of comparisons been shown in graphs, with galls on the ordinates and roots on the abscissas, the varieties would have been distributed in fair proximity to a diagonal line bisecting the angle formed at the origin of these axes. Thus a positive correlation is shown to exist between varieties in the number of non-terminal galls and root length, and a similar relationship appears when terminal galls and root numbers are compared.

The small number of plants representing each variety prohibits the use of Product-Moment correlation tables to determine the precise values of the aforementioned relationships. Nevertheless, the number of non-terminal galls, terminal galls, number of roots, and root lengths are all known to vary significantly for the several varieties, at least in many of the comparisons (table 6). Rank-Difference correlation may, perhaps, be used to express the presence of such relationships without necessarily indicating their extent. By this method, the correlation between number of non-terminal galls and length of roots, for the eight varieties is 0.95 $\pm$0.024. The correlation is 0.92 $\pm$0.038 when the number of terminal galls is associated with the number of roots possessed by each variety.

Varietal tolerance and root length reduction

We have concluded that, with a uniform inoculation, the different pineapple varieties have shown remarkable uniformity in the number of galls formed on each linear foot of root length grown by each variety. Table 6, however, makes it quite evident that certain varieties actually possessed many more galls than others at the end of 6 months' exposure to infection. Also, the varieties differ,

not only in number of roots, but in length of roots as well, according to Collins and Hagan (2). From this information, it might be determined whether nematodes cause a reduction in root length due solely to their abundance on the roots, or whether the different varieties of pineapples react differently to nematode attack and thus suffer in length of roots. If nematodes are entirely responsible for root length reduction then it might be expected that those varieties with the greatest number, or proportionally greatest number of galls, i.e. largest numbers of parasites, would suffer most. Varieties with smaller populations of the parasite should be less affected, and their root systems be less severely damaged. On the other hand, if plant response is the determining factor, then we would expect more resistant varieties to show less root loss than others, even when their roots bear large populations of nematodes. In other words, is degree of infection also a measure of damage when different varieties of pineapples are compared? Goodey (5) has shown that, within limits, an index of susceptibility could be determined and associated with a measure of plant injury in legumes attacked by *Tylenchus dipsaci*. That is, among his varieties, he could find some with light infections and with moderate damage to the plants. Another lot with heavier infections suffered more damage; whereas a third group, severely infected, showed greatest injury.

Perhaps one of the quickest methods of determining whether the nematode parasite or relative varietal tolerance of the host plant is the major factor in root length reduction is to employ the Rank-Difference correlation formula:

$$rho = 1 - \frac{6\ \Sigma D^2}{N\ (N^2 - 1)}$$

The probable error of each correlation may be ascertained by the following formula:

$$\text{P.E.}_r = \frac{.7063\ (1 - r^2)}{\sqrt{N}}$$

Both formulae are taken from Garrett (3). Obviously, infested roots alone cannot be considered, for it is entirely possible that uninfested roots might grow longer in order to compensate for the abnormal shortening of the others. Therefore, the correlations have been computed on the basis of average reduction in length per root for each variety.

When Rank-Difference correlation is ascertained for average reduction in length per root and number of galls per root, the value or $rho = 0.239 \pm 0.020$. The correlation in average number of terminal galls per root is 0.358 ± 0.045. Correlation based on the average number of non-terminal galls per root equals 0.143 ± 0.017. Since $N = 8$ the probable errors are quite apt to be larger than those given by the formula used. There is no correlation for the average number of galls per foot of root length as the answer is 0.048 ± 0.024; and a negative correlation appears to exist for total galls as $rho = -0.71 \pm 0.124$.

Apparently there is very little, if any, evidence in these data that the number of galls, terminal galls, galls per foot of root length, or galls per root, found in any variety, and associated with a definite amount of root length reduction in that variety, can be used to predict what will happen to any of the other varieties when attacked by nematodes. They have not responded alike, or the above correlations would be high, therefore varietal response must be intrinsic and different in value for the different varieties.

Nematode populations

Some consideration may now be given to the question of nematode populations. It is not yet known whether the same number of nematodes will produce galls of equal sizes on the different varieties of pineapples. No variety seemed to have a preponderance of galls of any one size in its roots in this study; therefore, the nematode population in the different varieties at the end of the experiment was assumed to vary, in general, according to the average number of galls present in each variety.

Of the factors favoring a rapid increase in the nematode population—Chapman's (1) biotic potential—only three are of special interest here. The first of these is the matter of fecundity. The egg masses used for the original soil inoculations contained, perhaps, about 500 eggs in each ootheca. Egg masses subsequently deposited within the pineapple root galls may have averaged the same, or approached this figure. Whatever the actual number may have been, one fact remains outstanding; that is, the rate of increase is very high. Actual field conditions seem to permit the same rate of multiplication.

Field observations have shown that several generations occur annually. Godfrey and Oliveira (4) have determined the minimum elapsed time for the life cycle in pineapple roots to be approximately 35 days. The plants used in this test were exposed to infection for 6 months which might permit of four or five generations forming the galls tabulated here. Under field conditions, the pineapple cycle usually covers 30 months and the number of generations is thereby considerably increased.

Temperature, too, has a very decided effect upon the length of the life cycle, and the number of nematode generations possible within a given period of time will be determined by this factor, if other conditions are uniform. Therefore, although this species of nematode continues its active development throughout the year in the Hawaiian Islands, population increase is much more rapid during the summer months.

The figures in table 3 may be interpreted from the viewpoint of increase in the nematode population. Cayenne and Hilo varieties have the same number of galls per plant, and significantly more than any of the other varieties. Lot 520, for example, may be considered to be quite superior to these two in having 20 galls less per plant 6 months after inoculation started. Some of the other varieties show even greater differences. It should be reasonably safe to assume that environmental resistance would be fairly uniform for all of the containers

except for one factor. This factor is the matter of difference in the number of roots, especially the fine lateral and fibrous roots, possessed by each variety Plants with an abundant supply of these roots, it is thought, should pick up more nematodes from the soil infestation, thus resulting in fewer large galls in the main roots. But there seems to be no such relationship between number of roots, nematode inoculation, and galls. Lot 520 has the greatest number of fibrous roots, Cayenne and Hilo next, and Ruby and Taboga have comparatively scanty root systems. Yet Ruby and Taboga show the fewest galls in the main roots, Lot 520 almost as many, and Cayenne and Hilo possess three times as many as the first two mentioned. The amount of inoculum added to the soil was not excessive, judging by field conditions, and none of the varieties of plants reached a saturation point in the number of galls that could be formed in the roots.

Apparently the two varieties being grown commercially in the Hawaiian Islands are contributing the largest number of galls from a given inoculation, at the end of 6 months of growth following inoculation. No doubt, the continued planting of Cayenne and Hilo varieties is an important factor in the production of large nematode populations in Hawaiian pineapple soils. It is an interesting speculation, whether Ruby, Taboga, Wild Kailua, or some other variety having comparatively fewer galls from a given inoculum, as shown by this test, would maintain the present high nematode populations that we find when Cayenne and Hilo are grown.

A possible solution of the nematode problem

The evidence already presented is believed to have shown that amount of infection and degree of root damage to pineapples are not highly, if at all, correlated when different varieties are compared. Tolerance or resistance to nematode attack is, therefore, presumably a physiological property of the plant variety. Should it later prove to be merely a physical stoppage of root growth, the value of the present discussion is not necessarily impaired, for the varieties do exhibit marked differences in the visible amount of reduction in root length due to nematode attack.

Cayenne and Hilo varieties are seriously curtailed in root development when nematodes are present in their roots. On the contrary, Lot 520 seems to show very little, if any, root loss due to their influence. Lot 520 is a hybrid whose male parent is Wild Brazil, a plant which appears to be quite tolerant to this species of nematode. The other parent is the commercial Cayenne, a much less tolerant variety. Evidently, tolerance is a genetic factor, or complex of factors, which distinguishes certain varieties from others.

Lot 520 is not a commercial type, but the triploid hybrids, having 50 Cayenne and 25 Wild Brazil chromosomes, show some promise of commercial value. Further, there is no reason to suppose that its tolerance to nematodes would be lost if it owes this factor to actual genetic differences in its chromosomes. Continued hybridization, especially back-crossing, may eventually produce a

highly tolerant, yet commercially desirable plant. Such an individual once obtained, either by these crosses or by others, would establish a clon whose perpetuation and extensive vegetative multiplication would greatly relieve the pineapple industry of the necessity of considering this parasite seriously.

SUMMARY AND CONCLUSIONS

Eight varieties of pineapples have been examined for numbers of galls produced in their roots by the nematode, *Heterodera radicicola*. None is immune to attack.

Comparisons are based on numbers of non-terminal, terminal, and total galls; also on galls per root, and galls per foot of root length. The numbers of significant differences in the 28 possible comparisons in each classification are as follows: for non-terminal galls, 9; terminal galls, 22; total galls, 20; galls per root, 7; galls per foot of root length, 4.

There are few significant differences in the number of non-terminal galls, but some varieties, as Natal, seem to continue root growth in spite of nematode attack, thus producing a comparatively large number of these galls. The actual number per variety appears to vary with root length. All but one of the significant differences agree with terminal gall differences in comparing the same varieties.

A terminal gall is formed when root growth ceases upon invasion of the root tip by nematodes. The different varieties vary greatly in the number of these galls upon their roots. The actual number in each variety seems to depend upon the number of roots possessed by the plant. There are, also, more significant differences in this classification than in the others. From 54 to 77 per cent of all galls fall into the terminal gall class, depending upon the variety.

Comparisons of total number of galls show 20 significant differences in this classification compared with 22 terminal gall differences. Nineteen of these duplicate terminal gall comparisons. In every terminal gall comparison, whether significant or not, any variety having more terminal galls than another also will be found to exceed the other in the total number of galls, except in the Natal-Pernambuco comparison where the former has fewer terminal galls, yet exceeds Pernambuco in total galls.

In number of galls per root there are 7 significant differences in 28 comparisons. Comparisons in number of galls per foot of root length show only four significant differences. These few differences emphasize the fact that, after all, the infection occurring in all varieties is noticeably uniform when the extent of the root systems possessed by each variety is taken into account. From this it follows that all varieties are about equally susceptible to attack, as those varieties having the greatest root lengths show more galls.

The different varieties apparently do not respond alike to nematode attack when Rank-Difference correlations are used to determine the correlation between average reduction in root length per plant and numbers of terminal galls, total galls, galls per root, or galls per foot of root length. At least such indexes of

correlation are very low. The amount of root length reduction expresses differences between varieties in their relative tolerance, or lack of tolerance, to nematode attack.

Some of the varieties developed three times as many galls within 6 months as did others. It is assumed that populations varied according to the number of galls which appeared in this length of time. Cayenne and Hilo bore the greatest number of galls at the close of the experiment. It would seem that these two varieties are an important factor in maintaining the present high nematode population in Hawaiian field soils.

Tolerant hybrids which show little influence of nematode attack have already been developed and it is thought that a possible solution of the nematode problem might come through the application of genetic principles in nematological investigation.

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THE ELECTROMETRIC DETERMINATION OF CHLORIDES IN SOILS BY THE SILVER-SILVER CHLORIDE ELECTRODE

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Heretofore, the determination of chlorides in soils has been made by the gravimetric or volumetric procedure, either procedure, however, necessitating the preparation of the soil extract.

The application of the silver-silver chloride electrode to the determination of chlorides offers the advantage of greater rapidity, in that the determination may be made on the soil suspension or extract. The turbidity factor and uncertainties attending the use of an indicator, as in the volumetric procedure, are also eliminated.

The present investigation presents a comparison of results obtained by the silver-silver chloride electrode and the silver chromate methods.

THEORETICAL CONSIDERATIONS

Adopting a procedure similar to that used by Cavanagh (2), Best (1) applied the Ag-AgCl electrode to the determination of chlorides in Australian soils using a quinhydrone electrode as a reference electrode, the pH value of which may be calculated as follows:

The equation for the potential difference of an Ag-AgCl electrode in a solution containing chloride ions is:

$$(A) \qquad E_{Ag} = E'_{Ag} - \frac{RT}{F} ln\ [Cl^-]$$

the constant E'_{Ag} having the value 0.2221 volts at 25°C. (5). The value of $[Cl^-]$ at the end-point in the titration of a chloride solution with silver nitrate is taken as 1.30×10^{-5} at 25°C. (4), namely, the solubility of silver chloride in water. Inserting these values in equation (*A*) gives for E_{Ag} the value 0.5109 volts at 25°C. If an Ag-AgCl electrode is in combination with a quinhydrone electrode of the same potential then at the end-point of the titration the potential difference between two such electrodes should be zero. The pH value of the buffer solution used as the quinhydrone electrode may be computed from equation (*B*) by equating to zero potential, or by substitution in the working equation for the quinhydrone electrode.

$$(B) \qquad E = E_0 - E'_{Ag} + .05912 \log [H^+]\ [Cl^-], \text{ at } 25°C.$$

The pH value of this buffer solution is calculated to be 3.18 for 25°C. The lower value, pH 3.03 at 25°C. calculated by Best (1), is due to the use of different values for E'_{Ag} and the solubility of silver chloride. If the buffer solution had a pH value of 3.4 at 25°C. zero potential would occur at pCl 4.66 instead of at the correct pCl 4.89. In 50 ml. the correct titre would necessitate the further addition of 0.025 ml., about one-half of a drop of $N/35.457$ silver nitrate. From Cavanagh's data (2) the pH value of the buffer solution at 16°C. is 3.25. It is evident that a buffer solution of pH 3.1 to 3.3 is sufficiently accurate.

With the addition of silver nitrate the potential decreases until at the end-point it becomes zero when with further addition of silver nitrate (within one drop) the polarity reverses.

METHODS OF EXPERIMENTATION

Preparation of the silver-silver chloride electrode

The Ag-AgCl electrodes designed by the writer were made by winding one end of a thin platinum wire about 3 cm. long around the end of a silver wire about 1 mm. in diameter and about 5 cm. long and heating the joint with a blast lamp until the silver wire just fuses to the platinum wire. The platinum end including the joint was inserted into a narrow glass tube constricted about one-half inch from the end and permitting the passage of the platinum wire up to the joint. The platinum wire was sealed in at the constriction so that a portion extended above the seal, permitting the customary mercury contact. About 3 to 4 cm. of the silver wire extended from the end of the tube and was sealed at this point with DeKhotinsky cement. The cleaned wire was coated with silver chloride by making it the anode in the electrolysis of a dilute solution, about 0.1 N potassium or sodium chloride, using a current density of 2 to 3 milliamperes per square centimeter for about one hour. The current was supplied by a 4-volt storage cell by the insertion of sufficient resistance. The used electrodes were cleaned with ammonium hydroxide and polished with a very fine abrasive, such as crocus cloth, and then thoroughly washed.

Preparation of the quinhydrone electrode

A KHPhthalate-H_2SO_4 buffer solution free from chlorides was used for the quinhydrone electrode and was prepared as described by Clark (3) but with the substitution of sulfuric acid for hydrochloric. The buffer solution has a calculated pH value of 3.18 at 25°C.; however, a pH value of 3.1 to 3.3 at 25°C. is sufficiently accurate. The quinhydrone electrode was prepared by adding about 0.05 gm. of quinhydrone to about 15 ml. of the buffer solution, in a test tube.

Apparatus

A platinum foil electrode and one end of a U-shaped agar bridge, held in place by a rubber stopper, made contact with the quinhydrone electrode.

The other end of the agar bridge passed through a rubber stopper holding the Ag-AgCl electrode. The agar bridge was made by heating a 5 per cent agar solution saturated with potassium nitrate, drawing into a dry U-shaped tube, and allowing to cool.

In the present work a potentiometer was used, the quinhydrone and Ag-AgCl electrodes being connected to the positive and negative terminals respectively. Prior to an electrometric determination it is advisable to check the system by titration of a definite volume of $N/35.457$ potassium chloride made up to 50 ml.

Procedures

When employing the electrometric procedure described by Best (1), the results obtained on some of the soils in table 1 were too high because of the drifting back of the end point. This necessitated further increments of silver nitrate in excess until the end point remained constant. In Best's procedure no mention is made concerning the reaction of the soil suspension. It was found, however, that unless the reaction of the soil suspension was adjusted to about pH 2.0 the results in many cases were in error. By adjusting the reaction of the suspension to about pH 2.0 any effect of carbonates on the titration is also eliminated. Constant stirring by an electric stirrer was found preferable to stirring with a glass rod after each addition of reagent as recommended by Best.

The electrometric procedure adopted is as follows:

To a 150-ml. beaker were added 5 gm. of soil, passing a 50-mesh sieve, and 50 ml. of distilled water. The sample was increased to 10 gm. for small amounts of chlorine and decreased for large amounts. When the soil extract was being used an aliquot was made up to approximately 50 ml. with distilled water. The Ag-AgCl electrode and agar bridge were allowed to reach nearly to the bottom of the beaker and the suspension was continually stirred by an electric stirrer during the determination. Sufficient sulfuric acid (1 + 2) was added dropwise to the suspension or extract to just redden a strip of thymol blue paper, about pH 2.0, and this reaction was maintained. At the end of 10 minutes the circuit was momentarily closed and the galvanometer deflection observed, from which an approximation may be had of the volume of $N/35.457$ silver nitrate required. During the course of a titration, particularly on the soil extract, it may be necessary occasionally to wash the electrode free from any adhering silver chloride. Silver nitrate is added, with subsequent adjustment of the potentiometer, until the galvanometer deflection just reverses, indicating the end-point. It is desirable to continue the determination 5 minutes longer to be sure the end-point is constant. The silver nitrate solution is made up as $N/35.457$ so that 1 ml. is equivalent to 1 mgm. of chlorine. An $N/35.457$ potassium chloride solution may be used for back titration if necessary.

For preparing the soil extract a soil-water ratio of 1 to 5 was shaken in a 250-cc. pyrex centrifuge bottle in an end over end shaker for 1 hour. The suspension was allowed to stand for a while to permit sedimentation and then filtered through a Mandler filter, care being taken to discard the first portion of the extract, because of the absorptive effect of the filter which causes a slight concentration change in the solution.

For the silver chromate method an aliquot was neutralized and titrated with $N/35.457$ silver nitrate, potassium chromate being used as an end-point indicator.

RESULTS

The results obtained by the Ag-AgCl electrode on the soil suspension and extract and by the silver chromate method on the extract are presented in table 1. These soils varied in reaction from about pH 3.4 to 10.5, and included peats, mucks, clays, loams and silty clay, clay, silt, and fine sandy loams from Arizona, California, Colorado, Indiana, Maryland, Missouri, New Jersey, New York, Ohio, South Carolina, and Texas.

With the exception of soil number 5 the agreement between the different methods is very good. In this instance the result on the suspension by the

TABLE 1

Chloride results on soils determined by the Ag-AgCl electrode and silver chromate methods

NUMBER	Ag-AgCl ELECTRODE		SILVER CHROMATE METHOD—EXTRACT	NUMBER	Ag-AgCl ELECTRODE		SILVER CHROMATE METHOD—EXTRACT	NUMBER	Ag-AgCl ELECTRODE		SILVER CHROMATE METHOD—EXTRACT
	Suspension	Extract			Suspension	Extract			Suspension	Extract	
	per cent Cl	*per cent* Cl	*per cent* Cl		*per cent* Cl	*per cent* Cl	*per cent* Cl		*per cent* Cl	*per cent* Cl	*per cent* Cl
1	0 004	0 004	0 004	17	0.043	0 040	0 042	33	0.175	0 170	0 175
2	0 006	0 005	0.005	18	0.044	0 042	0.047	34	0 185	0.180	0.185
3	0 006	0 005	0 006	19	0 042	0 046	0 048	35	0 220	0 215	0 215
4	0 006	0 005	0.007	20	0 055	0 052	0 056	36	0 241	0 239	0 243
5	0 017	0.008	0 009	21	0 072	0.069	0 069	37	0 244	0 248	0.251
6	0 009	0 008	0 009	22	0.072	0 068	0 071	38	0 273	0.268	0.271
7	0 018	0 015	0.016	23	0 072	0 069	0 071	39	0 256	0 270	0 275
8	0 016	0 016	0.018	24	0 080	0.081	0 084	40	0 325	0 325	0.329
9	0 021	0 019	0.021	25	0 085	0.081	0.085	41	0.417	0 412	0 414
10	0 028	0 027	0 028	26	0 082	0.083	0 086	42	0.454	0 450	0 455
11	0 033	0 027	0 028	27	0 119	0.117	0 121	43	1.298	1.282	1 290
12	0 034	0.031	0 032	28	0 126	0 127	0.129	44	1.419	1.410	1 415
13	0 034	0 034	0 035	29	0.144	0 138	0.144	45	1.493	1 502	1 515
14	0 036	0 035	0 035	30	0 147	0 149	0 152	46	1.574	1 588	1 609
15	0 036	0 038	0.040	31	0 150	0.152	0.151	47	2 083	2.090	2 100
16	0 039	0 040	0 042	32	0 168	0.165	0 168	48	2 104	2 121	2 143

Ag-AgCl electrode was higher than the Ag-AgCl electrode and silver chromate results on the extract, the latter two results, however, being in excellent agreement. During the titration of this suspension the galvanometer deflection, when adjusted to zero after silver nitrate was added, would drift back, corresponding to an increase in potential and indicative of an increase in the chloride-ion concentration.

A temporary end-point was reached but the deflection gradually reversed again, necessitating further additions of silver nitrate in excess of the correct titre until at last a constant end-point was reached. With this soil the tendency to drift was more pronounced without the addition of sulfuric acid.

Such a tendency for the galvanometer to drift was observed among some of the other soils in table 1, when the addition of sulfuric acid to the suspension was omitted, but by maintaining the pH value at about 2.0, the drifting was eliminated and the results were in good agreement. This tendency to drift, however, was not observed during the electrometric determination on any of the extracts. With soils such as number 5 where the electrometric result on the suspension was too high as indicated by the behavior of the galvanometer, the electrometric result on the extract, however, shows good agreement with the silver chromate result.

In regard to the effect of organic matter on the determination, Best (1) applied the method to Australian soils low in organic matter and to one soil of high humus content and found good agreement between the electrometric and

TABLE 2

Organic matter in soils

NUMBER	ORGANIC MATTER	NUMBER	ORGANIC MATTER	NUMBER	ORGANIC MATTER
	per cent		*per cent*		*per cent*
2	9 29	17	0 74	36	0 52
5	5 21	21	0 31	37	3 99
7	3 97	23	0 93	38	9 29
9	53.0	29	0 36	39	6 71
10	0.14	30	0 28	40	0 83
11	1 89	31	0 14	41	8 77
12	3 15	32	0 86	42	0 71
13	0 26	33	0 14	43	0 65
14	0 40	34	0 10	44	6 62
15	2 55	35	0 41	45	7 91

silver chromate methods. The organic matter[1] was determined on most of the soils in table 1, and the results are presented in table 2.

Two peat soils not included in table 1 showed very good agreement between the Ag-AgCl electrode and silver chromate methods. There is apparently no relationship between the anomalous behavior of soil number 5 and the total organic matter, in view of the fact that other soils in table 2 containing larger amounts of organic matter showed good agreement between the two methods. Soils number 5 and 7 were the same soils but number 5 had just been mixed for greenhouse use, whereas number 7 had been in use five or six weeks. The results in table 1 on number 7 are, however, in very close agreement. After soil number 5 was treated with about 30 per cent hydrogen peroxide, the drifting as previously described was absent during the electrometric determination and the result in this instance agreed very closely with the electrometric and silver chromate results, on the water-soluble extract, given in table 1.

[1] The author expresses his appreciation to W. R. Leighty of this laboratory for the organic matter determinations.

SUMMARY

The application of the Ag-AgCl electrode to the determination of chlorides in the soil suspension or extract is described.

Good agreement was found between the results obtained by the Ag-AgCl electrode method, on the soil suspension and extract, and the silver chromate method on the extract.

The advantages of the electrometric method are its rapidity and the elimination of the turbidity factor and of the use of an indicator.

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BASE INTERCHANGE INDUCED BY CALCIUM, MAGNESIUM, AND SODIUM NITRATES IN A 6-FOOT COLUMN OF SOIL-SUBSOIL

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It was shown by Mooers, MacIntire, and Young (3) that when heavy equivalent additions of sodium, calcium, and magnesium nitrates were made to a Cumberland clay loam, there was a marked difference in the speed with which the NO_3 ion passed through the 5-foot underlying depth of clay subsoil. The outgo of nitrates from the sodium nitrate addition was much more rapid than that from the equivalent amounts of either calcium or magnesium nitrate. The nitrate recovery was considered without reference to the cation combination. The present paper shows the base exchange that was induced by each of the three nitrates during a 9-year period of leaching by natural rainfall.

EXPERIMENTAL

Soil and placement. The leachates were from an 8-inch depth of soil underlaid by five 1-foot demarcated layers of a red clay subsoil in lysimeters of 12-inch diameter and 1/55,462-acre area. The surface soil was a Cumberland clay loam that had never been limed. It was a well-buffered soil, containing 22 per cent colloids. The pH values of the soil and subsoil were 6.84 and 6.40, respectively. The amounts of acid-soluble and exchangeable bases in both soil and subsoil are shown in table 1.

The tanks were filled December 15, 1921. A layer of gravel, topped by a layer of coarse quartz, was first introduced. Upon this was placed a 1-foot depth of $\frac{1}{4}$-inch sifted moist clay in two equal fractions. Fifteen tamps were given each successive 6-inch layer by means of a circular tamp that covered the entire subsoil surface. The successive 1-foot depths were separated by discs of asphaltum-coated, $\frac{1}{8}$-inch galvanized iron wire "cloth." The moisture-free weight of each soil charge was 39.27 pounds. The respective m.f. weights of the first- to fifth-foot layer of subsoil were, *52.26*, *52.23*, *48.21*, *48.39*, and *52.56* pounds.

Nitrate additions. The tanks were allowed to leach without additions until May 9, 1922, the rainfall during that period being 27.4 inches. The original objective was to determine the recovery and the speed of the movement of the nitrate ion through clay subsoil. Previous studies had indicated that the nitrate recoveries from sodium nitrate were obtained largely as calcium and

magnesium nitrates. These two nitrates were therefore run in parallel with sodium nitrate. The plan called for single equivalent additions of the three nitrates at the rate of 600 pounds of $NaNO_3$, the solutions of the three salts to be applied to the soil surface. Through an error in decimal place in the computations, the additions were made at the constant rate of 6,000 pounds of $NaNO_3$. It therefore seemed probable that treatments so heavy would effect exchange reactions of sufficient magnitude to register definitely in the percolates. The

TABLE 1

Acid-soluble and exchangeable bases in Cumberland loam and red clay subsoil, m. f. basis

COMPONENT	ACID SOLUBLE*		EXCHANGEABLE†	
	Soil	Subsoil	Soil	Subsoil
	per cent	*per cent*	*per cent*	*per cent*
CaO as $CaCO_3$ eq.	0.278	0.140	0.092	0.033
MgO as $CaCO_3$ eq.	0 657	1 041	0 011	0 023
K_2O	0 265	0 396	0 010	0 014
Na_2O‡	0 016	0 020	0 0013	0 0023

* HCl 1.115 digestion.

† Ca, Mg, and K, leaching with normal solution NH_4Cl; Na, leaching with 0.05 *N* HNO_3.

‡ Direct method, Caley and Foulk (3).

TABLE 2

Calcium, magnesium, and potassium interchange in a 6-foot depth of soil-subsoil as induced by single equivalent additions of nitrates of sodium, calcium, and magnesium during a 9-year period*

ADDITION	$CaCO_3$ eq., LEACHED, POUNDS PER ACRE SURFACE, FROM 8-IN. SOIL AND 5-FT. SUBSOIL					
	Calcium		Magnesium		Potassium	
	Found	Exchanged	Found	Exchanged	Found	Exchanged
None	989	...	540	...	118	..
Sodium nitrate	1,319	330	825	285	185	67
Calcium nitrate	1,862	873†	1,615	790	164	46
Magnesium nitrate	1,960	971	1,604	779†	156	38

* Constant of 6,000 pounds of $NaNO_3$.

† Derived from the addition.

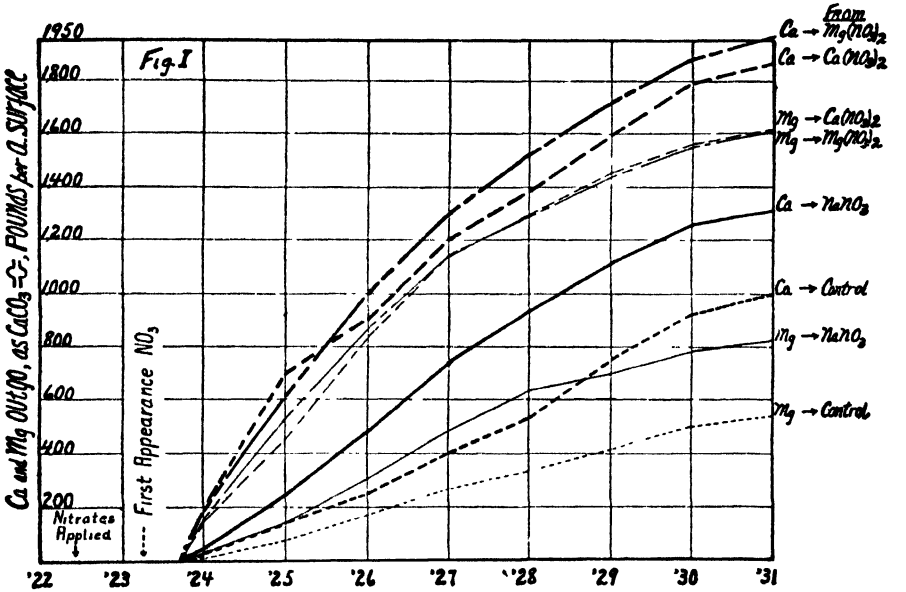
dissolved nitrate additions were made May 9, 1922, after a preliminary settling period of 145 days. Three tanks were used for the untreated control and three for each of the nitrate additions.

Ratio of rainfall to leachings. The rainfall for the 9-year period, May 9, 1922, to May 8, 1931, was 445.52 inches, or an annual average of 49.50 inches. The rainfall recoveries, as averages for the triplicated units for each treatment, were: control 64.18 per cent; calcium nitrate 64.88 per cent; magnesium nitrate 64.43 per cent; and sodium nitrate 65.98 per cent. The rapidity of the movement

may have been influenced by the specific treatment, but there was little difference between the ultimate amounts of the percolates.

Leachates. The first enhancement in nitrate outgo appeared in the percolates collected between January 3 and April 18, 1923. Beginning with the next collection, the outgo of calcium, magnesium, and potassium was determined. The totals of Ca, Mg, and K, obtained by averaging each 3-unit series, are given in table 2. The cumulative outgo for calcium and magnesium by annual periods is given in figure 1, in terms of $CaCO_3$ equivalence, pounds per acre.

Calcium outgo. The outgo of calcium induced by the magnesium nitrate addition was considerably greater than that found for the addition of sodium



nitrate. The enhanced outgo of calcium from the calcium nitrate addition was 98 pounds $CaCO_3$ = less than the amount forced by the magnesium nitrate from the column of soil-subsoil during the 9-year period. Considering the large factor used to convert the outgo from the small tanks to the acre-surface basis, the quantity of calcium recovered from the calcium nitrate addition and that exchanged by the magnesium of the magnesium nitrate addition may be considered as equivalent.

Magnesium outgo. The enhancement of magnesium outgo from the addition of magnesium nitrate is practically the same as that induced through interchange between the added calcium and the soil magnesium. The replacement of magnesium by the sodium of the sodium nitrate addition was decidedly less than the replacement shown for the calcium of the calcium nitrate addition. The reciprocal effect of the calcium and magnesium nitrates in liberation of

magnesium and calcium and the decidedly lesser activity of sodium nitrate demonstrate that the di-valent cations were much more effective than the mono-valent Na. It will be noted that the amount of exchangeable calcium in the surface soil is more than eight times that of magnesium, whereas the ratio in the subsoil is 1.45 to 1. The greater part of the exchanged magnesium was therefore probably derived from the 5-foot depth of subsoil.

Potassium outgo. The outgo of potassium from each of the three added nitrates was greater than that found for the three control tanks, but no extensive liberation of potassium was evidenced. The increases in potassium outgo were only small fractions of the liberations shown for calcium and magnesium, even though the amount of exchangeable K_2O in the surface soil was practically the same as that of MgO. The amount of exchangeable potassium in the subsoil was 61 per cent of the exchangeable magnesium. When considered as 9-year totals, the apparently greater activity of sodium nitrate in effecting potassium exchange is not convincing, and the enhancements in potassium outgo may be considered as in fair agreement for the three nitrate additions. The indicated exchanges must be considered as the total effect of the full 6-foot depth of soil and subsoil, so that a greater liberation of potassium in the upper zones may have been offset by a fixation in the lower zones. If this did take place the phenomenon was different from the effect registered by both calcium and magnesium.

With so small an exchange of potassium shown for the excessive additions of the three nitrates at the rate of 6,000 pounds of $NaNO_3$ ∞ with their mass-action capacity, it would seem that the actual liberation of potassium by economic additions of nitrates is not likely to be appreciable. This conforms to previous findings as to the activity of sulfates with the same surface soil alone (2) where 4,250-pound $CaSO_4$-equivalent additions of ferrous sulfate gave a marked enhancement in the outgo of sulfates of calcium and magnesium, with only a small increase in the amount of potassium leached.

Sodium outgo. Since a dependable direct method for the determination of sodium was not available when the experiment was begun, the difference between the total sulfates and the sum of the sulfates of calcium, magnesium and potassium was taken as the sodium sulfate equivalent of the sodium leached. The results so obtained were considered as qualitative, or at least only approximate, and they will not be given in detail. The direct determination of exchangeable sodium in the soil and subsoil by use of the sodium-magnesium-uranyl acetate method (1) shows the low occurrences of 0.0013 per cent and 0.0023 per cent for soil and subsoil, respectively.

If the sodium determinations be considered as only approximately correct, the increases found for sodium outgo from the additions of calcium, magnesium, and sodium nitrates were 2.8 per cent, 29.6 per cent, and 94.2 per cent, respectively. This greater outgo of sodium from the sodium nitrate addition fits in with the finding that the amount of calcium replaced by magnesium nitrate and

the amount of magnesium replaced by calcium nitrate were decidedly less than the replacements of calcium and magnesium shown for sodium nitrate.

SUMMARY

Triplicated single, heavy, equivalent additions of sodium, calcium, and magnesium nitrates were made to Cumberland clay loam underlaid by 5 feet of red clay subsoil, and the outgo of bases was determined annually for a period of 9 years in 12 lysimeters.

The replacements of calcium and magnesium by sodium were decidedly less than the reciprocal calcium-magnesium replacements shown for the two di-valent nitrates.

The amounts of calcium recovered from calcium nitrate and exchanged by the magnesium of magnesium nitrate were practically the same. Conversely, the recovered and exchanged quantities of magnesium from magnesium nitrate and calcium nitrate, respectively, were in close agreement.

The amounts of potassium replaced by the heavy additions of sodium, calcium, and magnesium nitrates were so small as to indicate that the amounts of potassium made available by ordinary amounts of nitrates would be inconsequential.

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SOIL ACIDITY AS A PHYTOPEDOLOGICAL FACTOR[1]

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The old Russian school of soil scientists thoroughly worked out the principles of genetic pedology based chiefly on morphological and but partly on chemical properties of soil horizons. In more recent years, as a result of investigations in the United States, in England, in Germany and elsewhere, on the rôle of microörganisms in the soil, an extensive and very fruitful biological trend was created in soil science, while by ascertaining the rôle and importance of the soil absorbing complex, a new branch of science, which can easily be termed soil physiology, has been created.

But the surface vegetation has been, so far, studied mainly by botanists, and consequently, its importance as a factor in soil formation has appeared to be of less significance than it really is. Because soils were studied independently from plants we failed to appreciate the laws governing the close relationship between plants and soil, which exists in nature.

And since all problems of agronomy may eventually be reduced to the question of the relationship between plants and soil, the importance of the said branch of science becomes most obvious.

Forest associations in virgin regions such as Siberia represent a most convenient object for the studying of this question. The author believes that the existence for many centuries of these associations on the same site, as well as their natural succession, would provide some indications whereby we could be guided in regard to those laws which form the basis of the mutual relationship of the plant and the soil.

In fulfillment of the program of work planned, the author contrived to collect from elevated forest regions on the eastern and southern coast of the Baikal Lake, a considerable quantity of soil samples of diverse horizons. These samples were used by the author during his studies on the relationship between Siberian forest associations and the soil.

After perusal of the literature, we have ascertained that, notwithstanding considerable research work in this connection, we may assume that only one fact has been proved, i.e., forest association stands succeed each other in a certain definite order.

It has not been possible, however, to establish the reason for such a sequence,

[1] Translated from the Russian by U. G. Andronikov.

or even to connect the character of the alteration of cover types with that of the alteration of soils.

As the author is not aware of any work dealing with all forms of acidity of soil profiles in connection with cover types, he has decided to determine, in numerous samples of East Siberian forest soils, not only the concentration of hydrogen ions but the exchangeable and hydrolytic acidities as well.

In these latter forms the author hoped to find a settlement of the question which was of interest to him, as these forms are more responsive than the pH to changes that take place in the soil.

The hydrolytic acidity is the weakest form of general soil acidity. When the exchangeable acidity is hardly detectable noticeable amounts of the hydrolytic form are always present. The deterioration of the soil affects, in the first place, the increase of the hydrolytic acidity.

A stronger form—the exchangeable acidity—arises in the soil with great difficulty. The exchangeable acidity being unchangeable, the increase of hydrolytic acidity shows that the process of soil oxidation is still in the first stage of its development. Under conditions of an anergetic and prolonged effect of the oxidizing factor, an increase both of the hydrolytic and the exchangeable acidity may be expected, with a noticeable decrease in the latter's value. Consequently, in various correlations of these values we may detect different rates of increase of soil acidity.

If we maintain that the hydrolytic form of acidity is its weakest form, we must recognize that the opposite process—the decrease of soil acidity—must again affect its hydrolytic form and alter the correlation of these forms, reversing them; that is, the exchangeable acidity being unchangeable, the hydrolytic form decreases. Under conditions of the stability of physico-chemical processes in the soil, the correlation of values of these forms of acidity may be more or less constant.

All the foregoing contentions, which are experimentally proved in this paper, have been taken by the author as a basis for his studies on the character of the effect of various tree associations on the soil.

METHODS OF DETERMINING SOIL ACIDITY

A 100-gm. sample of air-dried soil was shaken for 1 hour in a Wagner apparatus with 250 cc. of 7.5 per cent potassium chloride. The solution was then filtered and titrated with 0.1 *N* sodium hydroxide. A special 100-gm. sample was treated under similar conditions with a 13.5 per cent solution of sodium acetate. The concentration of hydrogen ions was determined in potassium chloride solution and, consequently, shows not only free hydrogen ions but those absorbed by the soil complex, which in this case are supplanted by potassium ions.

The acidity of the extract, determined by titration with KCl solution, we designate "exchangeable" acidity, and that with sodium acetate solution—"hydrolytic" acidity.

TABLE 1

pH values of the exchangeable and hydrolytic acidities of soil under various forest associations

Constant forms of phytopedological relations

NUMBERS OF SAMPLES	COVER TYPES	pH	EXCHANGEABLE ACIDITY*	HYDROLYTIC ACIDITY*	HORIZON	REMARKS
23	Fir-cedar	3 4	26 0	220 0	A	
29	Fir-cedar	3 6	88 4	154.7	A	
21	Fir-cedar	3.6	10 1	147 5	A	
22	Fir-cedar	3 9	54 2	107 5	A	
13	Fir-cedar	3 9	26 5	253 5	A	
17	Fir-cedar	3 9	21 1	109 7	A	
121	Fir-cedar	3 7	18 5	345 0	A	
115	Fir-cedar	4 0	26 6	116 5	A	
116	Fir-cedar	3 8	12 5	223 5	A	
35	Fir-cedar	3 7	32 0	160 2	A	
105	Fir-cedar	3 9	31 5	145 2	A	
Mean.		3 8	31 0	180	A	
23	Fir-cedar	4.6	10 6	32 3	B	
105	Fir-cedar	4 3	31 5	70 0	B	
119	Fir-cedar	4 1	58 0	93 7	B	
29	Fir-cedar	4 1	48 8	70 0	B	
Mean.		4 3	35 0	66 0	B	
1	Cedar-larch	4 1	32 0	136 0	A	
3	Cedar-larch	4 0	19 1	98 0	A	
61	Cedar-larch	4 1	60 0	192 0	A	
62	Cedar-larch	4 2	43 2	115 5	A	
4	Cedar-larch	3 9	26 0	89 0	A	
Mean.		4 1	36 0	126 0	A	
1	Cedar-larch	4 2	14 0	44 0	B	
3	Cedar-larch	3 8	37 0	55 7	B	
4	Cedar-larch	3 8	34 0	62 0	B	
61	Cedar-larch	4 0	77 2	108 0	B	
62	Cedar-larch	4 1	36 5	88 0	B	
Mean.		4 0	39 7	71 5	B	
46	Fir	4.3	16 5	44 0	A	
68	Fir	4 2	12 0	179 0	A	
112	Fir	4 5	6 0	175 0	A	
Mean.		4 3	11.5	130 0	A	
46	Fir	4 8	5 5	22 0	B	
68	Fir	4 9	1.5	60 2	B	
112	Fir	4.6	23.2	69.2	B	
Mean.		4 8	10 0	50 5	B	

* In this table and in tables 2 and 3, the values of the exchangeable and hydrolytic acidity are given in cubic centimeters per 100 gm. of soil and are results of the first titration without multiplication by coefficient.

TABLE 1—*Continued*

NUMBERS OF SAMPLES	COVER TYPES	pH	EXCHANGEABLE ACIDITY*	HYDROLYTIC ACIDITY*	HORIZON	REMARKS
24	Cedar	4.4	16.7	39.5	A	
25	Cedar	4.2	12.7	75.7	A	
48	Cedar	4.4	8.0	116.7	A	
102	Cedar	4.6	4 5	176 0	A	
103	Cedar	4.9	1.7	92.5	A	
104	Cedar	4.3	5.7	145.5	A	With birch
124	Cedar	4 2	15.7	293 0	A	With fir
127	Cedar	4 2	51 2	127 0	A	With A_0
49	Cedar	4.8	3 5	93 5	A	
Mean.....		4.4	12.5	128.8	A	
127	Cedar	4.6	15.7	51.5	B	
49	Cedar	4 3	12 0	34 0	B	
25	Cedar	4 6	8.2	31.7	B	
Mean.....		4.5	12 0	39 0	B	
52	Birch	5 5	2 0	91 0	A	
53	Birch	5 1	1 2	93 0	A	
136	Birch	6 0	1.7	80 0	A	
45	Birch	5.9	1.0	85 0	A	
Mean.....		5 6	1 5	87.0	A	
45	Birch	5.4	1.7	25 5	B	
52	Birch	5.4	7.5	38 5	B	
53	Birch	5 4	9 5	37 5	B	
Mean.....		5 4	6 2	33 8	B	
75	Aspen	6 6	0 2	13 8	A	Shady aspen
76	Aspen	6 3	0 4	11 8	A	
134	Aspen	6 1	0.8	61.0	A	
133	Aspen	5.5	1 5	67.5	A	Young aspen grove
Mean.....		6.1	0.7	35.0	A	
134	Aspen	6 6	10 0	32 5	B	
75	Aspen	6.7	1.0	3.7	B	Shady aspen
76	Aspen	6.5	1.0	7 0	B	
Mean.....		6 6	4.0	14 4	B	
57	Pine, larch	6.2	0 4	30 4	A	
58	Pine, larch	6 2	0.7	51.7	A	
82	Pine, larch	6.4	0.3	19 2	A	
26	Pine, larch	6 3	0 5	32 7	A	
32	Pine, larch	6 3	0.5	30.9	A	
Mean.....		6 3	0.5	33.0	A	

TABLE 1—*Concluded*

NUMBERS OF SAMPLES	COVER TYPES	pH	EXCHANGEABLE ACIDITY*	HYDROLYTIC ACIDITY*	HORIZON	REMARKS
128	Poplar	6.6	2 0	26.5	A	
128	Poplar	6 5	0 75	4 7	B	
77	Larch-pine	6 7	0 5	11 0	A	
79	Larch-pine	6 6	1.5	12 0	A	
Mean.....		6 6	1 0	11.5	A	
77	Larch-pine	6 4	0.75	6 75	B	
79	Larch-pine	6 7	1 0	5 5	B	
Mean.....		6 6	0 8	6 1	B	
55	Pine	6 7	1 5	6 5	A	
55	Pine	6 4	1 0	17 5	B	
57	Pine	6 3	2 5	10 5	B	
Mean..		6 4	1 7	14 0	B	

The concentration of hydrogen ions was determined colorimetrically, according to Michaelis' method.

The results of our studies on the acidity of soils in connection with cover types growing on them are shown in tables 1 and 2.

From these data we are justified in drawing a general inference that each type of associated forest tree is in strict relationship with the soil on which it grows. The relationship is mutual, i.e. one should not imagine that cover types use only types of soils, or varieties, formed under the influence of geological, climatic, and other conditions not of a biological character.

The soil on which a certain cover type grows is a direct result of the latter's life activity,—a resultant of the effect of centuries upon the mineral substrate.

Genetic pedology usually understands under the idea "forest soils" only a podzolization process, which is far from corresponding to the variability which we have found in the physico-chemical condition of the soil under various cover types.

Each forest association has its specific effect upon the soil and creates a special type, or rather, a biotype. We find it, therefore, necessary to introduce a concept in which there should be embodied not only any one particular type of soil but also the type of vegetation which formed it. These shall be "phytopedological classes," which comprise definite groups of associations. Each phytopedological class, as well as the groups of which it consists, possesses its own class index.

Our conviction that the principles by which we were guided in proposing the introduction of a phytopedological system are correct, was fortified, especially when, after computing table 3, we applied it to the analysis of certain phenomena. Some soil samples among those we had studied could not be classed according to our standards; however, when we applied a phytopedological interpretation to them, the discrepancies in acidity values were definitely explained.

TABLE 2

Transition forms of phytopedological relations

NUMBERS OF SAMPLES	COVER TYPE	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY	HORIZON	REMARKS
31	Pine-larch	5.2	6.5	37.5	A	Old burned area
73	Pine-larch	5.0	0.2	13.7	A	Thin forest
71	Pine-larch	5.8	0.9	70 5	A	Underbrush of cedar and pine
83	Pine-larch	4.5	4.5	119.0	A	Near 82
78	Larch-pine	6.5	0.5	90.0	A	Near 77
81	Larch-birch	6.2	1.1	87.0	A	
74	Larch-birch	5.4	9 0	129.2	A	
79	Larch-birch	3.8	46 6	133.5	A	Young stands
66	Larch-cedar	5.4	2 0	56 0	A	With A_0
60	Larch-cedar	4.5	4 0	90.0	A	
50	Larch-cedar	4.3	33 0	68 7	A	With fir
51	Larch-cedar	6 0	1.2	64.5	A	
51	Larch-cedar	4.2	29.2	51 5	B	
19	Cedar-poplar	4.0	18.5	117.2	A	
19	Cedar-poplar	4 9	2.5	18.2	B	
20	Cedar-poplar	4 4	10.0	24.3	A	
20	Cedar-poplar	4.3	31.2	67.2	B	
130	Poplar-cedar and fir	5 4	6 0	258 0	A	
64	Cedar-fir	6 1	2 0	116.0	A	With birch

TABLE 3

Phytopedological system

	COVER TYPE	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY	CLASS INDEXES OF ACIDITY			CLASS
		1	2	3	1	2	3	
1	Fir-cedar	3.6	18.0	238.0	.....	.....	.....	.....
2	Fir-cedar	3.8	42.0	132.0	3 7	30 0	185.0	I
3	Cedar-larch	4.1	36.0	126.0	4.1	36.0	126 0	II
4	Fir	4 3	11.5	130 0	.....	.....	.....	.....
5	Cedar	4.4	12.5	128 0	4 3	12 0	129 0	III
6	Birch	5.6	1.5	87.0	.....	.....	.....	.....
7	Aspen	6.1	0.7	35.0	.....	.....	.....	.....
8	Pine-larch	6.3	0.5	33 0	5.6–6 5	1.1	11.0–87.0	IV
9	Poplar	6.6	2.0	26.5	.....	.....	.....	.....
10	Larch-pine	6 6	1.0	11.5	.....	.....	.....	.....
11	Pine	6.7	1.5	6.5	6.7	1.5	6.5	V

SUCCESSION OF ASSOCIATIONS AND THE PHYTOPEDOLOGICAL SYSTEM

All geographers, in treating of the succession of cover types, mention the pine, birch, and aspen as being first to follow fires, to grow on denuded soils, and cite them, in general, as a pioneer genus; while the cedar and fir are claimed by all to be species completing the process of the succession of cover types. The results given in table 3 show that the class indexes of acidity of these groups of cover types are found at the extreme points of the pH range.

From the viewpoint of the phytopedological system the fact, recorded by botanists-geographers, of the extraordinary vigor of the larch, which occurs together with representatives both of the first and last stages of cover-type succession, becomes quite clear.

Our table also shows a combination of the larch and pine, growing on neutral soils of pH 6.6, and the cedar, dwelling on very acid soils with a pH value of 4.1.

Certain authors, after claiming the aspen and birch to be the first species to follow forest fires, mention the larch as coming next and then the pine. Reverting to our system we note that the larch actually connects a class, comprising the birch and aspen, with that of pure pine.

Following the idea that the character of cover types determines the amounts of soil acidity, and that a gradual succession of cover types brings about an adequate alteration in pH values, we have attempted to establish, on the basis of analysis of horizon B, the trend of the said process in the past.

Let us examine, from this point of view, a few samples of soils:

NUMBERS OF SAMPLES	COVER TYPE	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY	REMARKS
82	Pine-larch	6 4	0.3	19 2	On slope
83	Pine-larch	4 5	4 5	119 0	Dell

Sample 83 was extracted from the same place as sample 82 only on a little dell. A greater moisture and a thicker cover of moss on the spot from whence the sample was taken, tended to increase the acidity. According to our phytopedological system, sample 82 belongs to class IV, whereas sample 83, because of its hydrolytic acidity, may be ranked in class III; its exchangeable acidity, however, having outgrown the limits of class IV has, nevertheless, by no means attained the value of class III. Such a condition may be designated as IV → III, i.e. as a transitory stage towards an increase of acidity under the local influence of a lowering of the relief and of the process itself being not in its first stage. These will be intraclass soils of the phytopedological system.

Let us analyze a few more samples:

NUMBERS OF SAMPLES	COVER TYPE	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY
71	Pine-larch	5.8	0.9	70.5
124	Cedar	4.2	15.7	293.0

Sample 71 should be, according to the nature of the cover type, classified in group 8; class IV and its exchangeable acidity actually correspond to indexes of that group. The hydrolytic acidity, however, is considerably higher and corresponds, approximately, to group 6. Evidently, this is the first stage of the alteration of the character of the soil, namely, a condition IV → IV, 6, that is, an increase of acidity beginning with an increase of its hydrolytic form.

Sample 124, according to its cover type, should belong to class III of our phytopedological system. The hydrolytic acidity of the sample is, however, considerably higher. Referring to the diary we find a remark, viz.: "admixture of fir." The fir belongs to class III, but in combination with the cedar it forms the highest form of class I acidity; therefore, this correlation of the given association and the soil may be designated thus: III → I. When it is considered that the pH values and the exchangeable acidity almost entirely correspond to class III, it should be recognized that we actually have only the initial stage of the process, which as yet has had no effect upon the exchangeable acidity.

An interesting example of the progression of soil acidity is obtained from a comparison of the two soil samples shown:

NUMBERS OF SAMPLES	COVER TYPE	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY
128	Poplar	6 6	2 0	26 5
130	Poplar with cedar and fir	5 4	6 0	258 0

We have here extreme specimens of the phytopedological belt of cover type, and the nature of the effect of the cedar-fir association on almost neutral soils of the poplar association is made apparent even more sharply; namely, the increase of the exchangeable acidity in sample 130 being very small, its hydrolytic acidity, as compared to sample 128, gives a marked increase. The phytopedological sign for the condition of sample 130 will be as follows: IV → I.

All these examples are samples of *progressive forms of soil acidity.*

We should include in the regression forms the whole group of the cedar and larch association, because, in this case, we detect, together with the exchangeable acidity, which is inherent in the higher class, the hydrolytic acidity of the lower class.

It would be more correct to consider this group, not as an independent class, but as an intraclass group playing a part in the regression of soil acidity, which has attained its highest limits. A further progressive increase of acidity has become impossible because the biological, or perhaps even the physico-chemical limit, has been reached, and the larch promotes an inverse process. That this particular phase is the beginning stage of "deoxidation" can be seen from the fact that the exchangeable acidity has not lost the values of its initial class I, while the hydrolytic acidity decreases to class III. Dynamically this group may be designated thus: I → III.

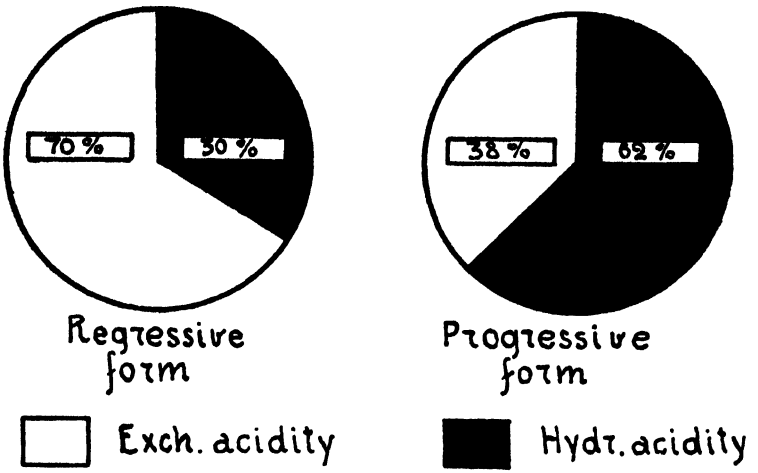

FIG. 1. CHANGES IN VARIOUS FORMS OF SOIL ACIDITY IN A CEDAR-PINE FOREST SOIL

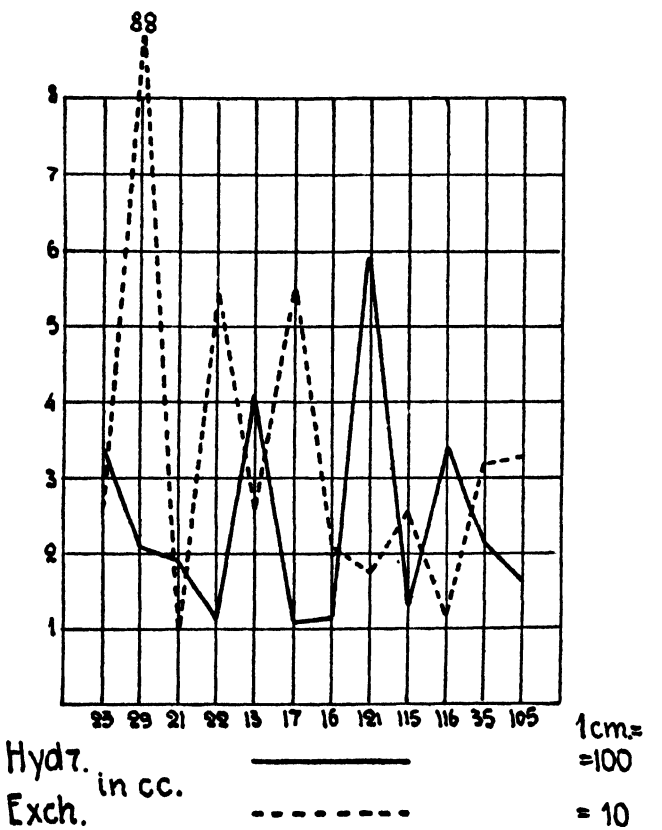

FIG. 2. PROGRESSIVE CHANGES IN HYDROLYTIC AND EXCHANGE ACIDITY IN THE SOIL OF A CEDAR-PINE FOREST ASSOCIATION

A closer perusal of figures obtained on the cedar-fir association, especially of their graphic rendering, has led to a very interesting observation. Both in our first work on the study of the acidity of taiga soils, and in the majority of data set forth in the present paper, we noticed a very distinct parallelism in the values of the exchangeable and hydrolytic acidities. Indeed, when soil samples were selected at random the exchangeable acidity was found to be directly correlated with the hydrolytic form. But, upon being selected in the same way, the cedar-fir association showed results diametrically opposite to those of the soil. All soil samples of the cedar-fir cover type, almost without exception, showed a decrease of the hydrolytic acidity when the exchangeable acidity increased, and vice-versa.

This extraordinarily interesting phenomenon prompted us to sum up the data on amounts of acidity contained in samples with prevailing exchangeable acidity, separately from those in which the hydrolytic form predominated.

Soil acidity of the cedar-fir cover type

	I CLASS	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY
1	Progressive form	3 6	18.0	238 0
2	Regressive form	3 8	42 0	132 0

This table clearly sets out two trends in the dynamics of the acidity of class I, namely, the progressive and the regressive forms. When the exchangeable acidity is at its highest (42.0) a decrease of the hydrolytic acidity to values of class II (132.0) indicates regression. On the other hand, the value of the hydrolytic acidity (238.0) in another group of samples denotes an opposite process of a progressive accumulation of acidity, judging by the exchangeable acidity, which has not yet reached its highest point. In this manner, the process of an inverse "deoxidation" under a cover type of cedar and fir, takes place within the area of said cover type, both with the participation of the larch and without it. Most probably the regression of acidity in this, as well as in other cases, means that the given cover type begins to deteriorate after reaching its limits of growth ability.

CYCLICITY OF PHYTOPEDOLOGICAL RELATIONSHIP

Referring to bibliographical data, we wish to draw attention to one fact, which from our viewpoint is very important in establishing principles of the evolution of associate vegetation.

Sukache (38, 39) finds that the greater part of birch-aspen and pine associations are not permanent, but temporary. After fires they are succeeded by the fir, and after destruction of the latter, pine growth recurs, either directly after the fir or after the birch.

Dokturovsky (12) ascertained that nowhere have associations of birch or of larch-aspen-birch any special herbaceous growth peculiar to them. It is for

the major part a mixture of specimens of pine, fir, meadow, and other associations, which indicates their temporary, transition character.

Borovikor (5) found that the area of ancient taiga (Siberian vast forests) of the Enissey province (cedar-fir) was almost entirely substituted by pine forests, as a result of fires. He describes such a consecutive succession as follows: Following a fire, when all vegetation, including herbaceous species and moss growth, is destroyed, the first to spread are the aspen and birch and next the larch and pine. Eventually the pine supplants all other species, leaving individual specimens of the larch, birch, and aspen. At this particular time taiga specimens, namely, the fir and cedar, recur.

Kouznetsov (27) states that the larch is to be found in any type of forest, including pine groves and the fir-cedar associations. The author finds that the groves are displaced by taiga species, through the larch, which eventually develops into an association of larch-fir and cedar.

M. Iliin considers that the birch and aspen are first to capture land after fires. The aspen occurs on more humid soils; the birch, on those more dry.

Thompson (40), after analyzing the pollen of 16 lake and swamp profiles, established the succession of cover types for the entire period after the ice-age in Esthonia. He distinguishes the following periods:

The birch prevails, pines and willows still occur The fir is absent.

The pine prevails instead of the birch, the latter attains a dominant position on very fertile soils only, it is less widely distributed, however, than the pine. The fir is absent.

Broadleaf forests (elm, linden, oak) are prevalent. The fir appears.

Broadleaf types recede. The fir attains its first maximum distribution.

Broadleaf cover types begin to vanish. The fir definitely invades the whole country.

In conclusion, Thompson remarks that in most upper zones the fir again recedes and the pine and birch play a more prominent part once more.

In comparing this consecutive succession (birch-pine-fir-birch), covering a period of many thousand years of forest life in Esthonia, with the aforementioned data submitted by Russian botanists in regard to the succession of forest associations after comparatively recent fires in Siberian forests (birch-larch-pine-fir-birch), the affinity of both these processes, which differ in regard to the range of time and place, suggests itself.

There is a definite sequence in the succession of forest associations. Forest fires, which have become more frequent since the appearance of man, tend to increase a rapid succession of forest associations, but do not interfere with the consecutive order of their occurrence.

As a rule the birch appears first and the further succession proceeds in two directions, namely the birch is either again directly succeeded by the cedar and fir, or it is first supplanted by the pine, which is followed by a return of the original taiga type. In other words the succession of cover types rotates within a small and a large circle.

If we apply this idea to the theory we are developing it will ensue that the birch may create such values of soil acidity as will be in conformity with the

requirements of the cedar and fir types, provided conditions of soil moisture are especially favorable. Under less favorable conditions the birch restricts the soil reaction to certain limits, which do not, however, attain minimums required by class I associations. This process is completed by the pine.

We have, however, to deal with the following situation: the succession of the birch by the pine in the phytopedological system is not a process of approaching, but rather of drawing away from, that condition of soil reaction which corresponds to the cedar-fir class.

The established opinion that a certain vegetation association requires identical soil conditions irrespective of its age is contradicted by the mere fact that associations such as cedar-fir, which in their mature state withstand especially high amounts of soil acidity, are reproduced under cover of pine and birch associations, which grow on soils having a reaction close to neutral.

We believe that after the soil supports a certain association for a long time, its reaction represents a final result as to limits of its effect upon the soil. These are extreme conditions which a given association can withstand and are not, by any means, always optimum conditions. Therefore, the expression generally used, that such an association "prefers" such soils, calls for a very serious correction. In the majority of cases it would be more correct to say that such an association "withstands" such soil conditions.

From this point of view we must assume that the fir and cedar are reproduced on soils with a neutral reaction and, with age, greatly increase the acidity; whereas the pine dwells on acid soils, neutralizing the reaction. Therefore the highest points of alkalinity for the pine and of acidity for the cedar and fir, according to our phytopedological system, should not be understood as the most favorable, optimum reactions for their "growth," but as a limit of alkali content allowing the "residence" of the pine and the limit of acidity for the "residence" of the cedar and pichta.

Optimum conditions of "growth" for the pine are to be found, in a literal sense of the word, in class I of the cedar and fir; whereas for the cedar and fir, optimum conditions exist in class V of the pine, which is in complete conformity with the aforementioned consecutive succession of these associations.

Sukachev (38, 39) states that the birch, oak, and probably the majority of broadleaf types are not, as a rule, reproduced under their own cover. Should optimum conditions of growth exist where an association grows, this phenomenon would contradict itself. It becomes clear, however, if our opinion, as set forth, is recognized as correct. In confirmation of this theory we can mention data obtained by Kouznetsov (27), who studied the artificial planting of pine in Western Siberia on chernozems, solonetz, and podzols. He found that pines planted on solonetz or slightly podzolized chernozems, perished. *He noted the most vigorous growths of pine on podzols* (7.3 m. elevation), the next vigorous on chernozems (5.6 m. elevation), and the weakest on solonetz (3.4. m. elevation). The bulk of the pine trunk on solonetz is almost 7 times smaller than that of a tree trunk on podzols.

Bearing in mind that soils on which mature pines generally occur are neutral and saturated with bases, we should anticipate a reverse phenomenon, provided we acknowledge that soils on which a certain association grows have a constant reaction. Hence, the pine begins its development on unsaturated, acid soils, and during its life cycle brings about a decrease in the acidity of the soil. Referring to the succession of the oak by the fir, Morozov (33) states that a litter of fir needles forms an acid humus, and a litter of oak leaves—a mild humus; and as the oak apparently is not reproduced under its own cover but only under that of the fir, we are dealing with an analogous instance of the succession of two types belonging to extreme classes of the phytopedological system.

Different genera differ in the nature and extent of alteration of the reaction. Such types as the larch and probably the fir and cedar may serve as examples of wider changes; and the birch—as one of restricted limits of acidity, within which they can possibly exist. We have given an example of a direct succession of the poplar (class IV) by the cedar and fir (class I). Hence, the alteration of soil reaction may be produced mutually by two types, or by a consecutive succession of several types, within a small and a large circle.

We believe that in various soil requirements of young and mature plants lies the solution of a number of phenomena which take place in forest life, and, in particular, in the mutual substitution of two types in the course of the succession of associated cover types. One of these, by providing remains of vegetation prepares the soil for the growth of young species of another genus, which in its turn, after having attained a dominant position, creates soil conditions imperative for the reproduction of the first genus. The phytopedological system is cyclic and is well balanced biologically.

FOREST LITTER AND HUMUS AND THEIR RÔLE IN FOREST REVEGETATION

Our object is not to discuss at length the very complicated question of the structures of soil humus. We shall quote only the most characteristic recent theories. Waksman's theory occupies the first place. He uses the word *humus*, rightly, only in quotation marks. By a systematic study of the decomposition process of various remains of vegetation, he proved that the so-called humus does not comprise any specific "humus" matter, but is composed of ordinary components, such as cellular tissue, lignins, and bacteria. In coöperation with other authors he found that the rapidity and the character of the decomposition of vegetative refuse, and consequently of the humus, depend first and foremost upon the nature and the age of the plant (41).

Pronevich (35), on studying the acidity of forest litters, gave the values shown in table 4 for different cover types.

We did not use for our studies pure needles or leaves, but the actual forest litter collected at one particular time and from soils under study. This resulted, therefore, in considerable fluctuations in the acidity values contained in litters of different cover types. However, our data show that the characteristics of

forest litters of diverse cover types differ, hence the further process of the litter's decomposition together with its final effect must needs differ.

TABLE 4

Acidity of needles and leaves

COVER TYPE	HORIZON A	HORIZON B
	pH	*pH*
Picea excelsa	4.3	4 6
Pinus silvestris	5.1	5 6
Betula pubescens	5 7	6 0
Acer platanoides	6 1	6 3
Tilia cordata	6 1	6 3
Alnus incana	6.4	6 6
Populus tremula	7 0	.

TABLE 5

Acidity of horizon A_0 of forest soils, Baikal District

NUMBERS OF SAMPLES	COVER TYPE	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY
15	Fir-cedar, pure fir,	3 7	49 2	253 3
14	and pure cedar	3 4	73 0	509 3
36		4 1	15 5	201 5
126		5 7	3 0	146 0
117		4 8	27.0	280 0
18		4.7	16 5	275 0
8		3 8	14 0	375 0
17		5 0	4 0	169 0
47		4.1	14 3	144 7
125		4.7	5 0	220 0
21		6 4	14 0	276 0
22		5 9	14 0	276.0
29		3.8	30.5	356 0
Horizon A_0, mean		4 6	21 0	265 0
Horizon A, mean		4.1	18.0	146 0

TABLE 6

Acidity of horizon A_0

NUMBERS OF SAMPLES	COVER TYPE	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY
75	Aspen	6 2	2 0	14 0
54	Aspen	6.5	3.5	55 0
128	Poplar, willow	6.7	2.0	26.5

It should be noted that while the exchangeable acidity of the forest litter and of horizon A may be considered equal, the hydrolytic acidity of the forest litter considerably exceeds that of horizon A, underlying it. Hence, the forest

litter affects the trend of the soil formation process through the medium of the hydrolytic acidity it creates. This is in conformity with the aforementioned effect on the soil of the organic matter contained in stable manure.

On comparing values of the exchangeable and hydrolytic acidities, in accordance with our phytopedological theory, we must recognize that we have a total progressive degree of acidity for the given samples.

The acidity of the aspen and poplar litter layers is comformable to the character of the effect upon the soil and to the place these cover types occupy in our phytopedological system.

We have purposely set forth in table 7 various cover types having the larch as their associate member. This plant is one of the most interesting from a phytopedological point of view. It is connected with soils fluctuating from 3.2 to 6.7 pH, and of hydrolytic acidity fluctuating from 12.0 to 457.0. Such an exclusively indifferent attitude of the larch toward the soil reaction, permits its incidence in any and all forest associations and it probably plays the part of a phytopedological "buffer." By discussing the effect of A_c on A we have

TABLE 7

Acidity of Horizon A_0

NUMBERS OF SAMPLES	COVER TYPE	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY
65	Pine-larch	6 7	1 0	46 2
79	Pine-larch	6 6	1.5	12 0
74	Larch-birch	5 4	9 0	129 0
50	Larch-cedar	3 2	20 0	475 0

raised a new question, that of a corresponding effect of horizon A on B. Any changes which have taken place in the character of surface vegetation must needs be reflected, by means of a consecutive effect, on all horizons. And, very likely, lower horizons may tell us more about the past condition of their surface vegetation than upper horizons, which have just been formed. Considering horizon B to be a "paleontological" formation, we shall attempt to apply this viewpoint to data we have on hand.

NUMBERS OF SAMPLES	HORIZONS	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY	COVER TYPE
51	A	6.0	1.2	64.5	Larch
51	B	4 2	29.2	51.5	Cedar

We picture the evolution of the given sample's soil formation process and the character of the participation therein of cover types, as follows:

In the past (see horizon B) this soil belonged to a higher phytopedological class than is seen from horizon A. The exchangeable acidity of horizon B—indubitably of a more ancient

origin than horizon A—is equal to the index of the cedar-fir association, i.e. to class I; at the same time the hydrolytic acidity recedes to class IV. Such a divergency speaks for the invasion of an association having lower indexes of acidity, and, as is usually the case, the hydrolytic acidity is first to diminish.

Horizon A may be ranked in the fourth phytopedological class, in accordance with values of its phytopedological indexes. Hence, in this case, we witness a completed process of the invasion by the larch of areas where the cedar formerly prevailed. This fact is supported by comparative data referring to the condition of forms of acidity of horizons A and B.

Let us make a few more excavations with a "paleontological" object.

NUMBERS OF SAMPLES	HORIZONS	pH	EXCHANGEABLE ACIDITY	HYDROLYTIC ACIDITY	COVER TYPES
19	A	4 0	18.5	117.2	Cedar-poplar
19	B	4.9	2.5	18 2	Cedar-poplar
20	A	4 4	10 0	24 3	Cedar-poplar
20	B	4 3	31 2	67 2	Cedar-poplar

Sample 19 in horizon B bears a convincing proof of the fact that the poplar dwelt here in the past, as all indexes of acidity of the given horizon are close to the normal indexes of class IV. Horizon A of this sample, however, even at this point, bears indexes of class III, showing an almost complete invasion by the cedar of the poplar. This may be designated as IV ↑ III.

Sample 20 illustrates an opposite phenomenon. In this case horizon B shows a greater acidity than horizon A; its exchangeable acidity pertains to class I, but a considerable decrease of hydrolytic acidity, as compared to the normal value of class I, testifies to its regressive movement having begun. This diagnosis is confirmed by examining horizon A, where the exchangeable acidity pertains to class III and the hydrolytic to class IV, as even in this case the decrease of hydrolytic acidity values, as against the normal value of its class, testifies to the beginning of a transition movement from class III into class IV. In other words phytopedological indexes of both horizons show an invasion of the cedar by the poplar. This phase will read: III ↓ IV.

These samples are of special interest because they represent, for the same type of association, inverse trends in the dynamics of acidity for each horizon. And, if figures of the acidity of these samples were to be considered from a descriptive point of view, they would only testify to an absolutely promiscuous distribution of all forms of acidity in each horizon even under the actual influence of one particular cover type. We should have been compelled to acknowledge, in regard to sample 19, that the podzol horizon rests on the surface of the soil.

Only a physiological outlook on facts has enabled us to understand processes taking place in the given samples, to determine the stage at which their development has been checked at this particular time, and even to foretell their future trend.

PHYSIOLOGY OF SOIL PROCESSES

The studying of soil from a physiological point of view has necessitated a few adjustments in the understanding of processes of horizon formation, dealt with in literature by the Russian school of soil scientists.

Soil horizons of the classical Russian school of pedology are a dead matter to the author in his capacity of physiologist. Examples discussed in the foregoing show that soil horizons are living biological formations, individually outlined, fighting for their existence, perishing, and regenerating. They bear traces of these processes as paleontological impressions.

If pedology studies horizons as morphological formations, the physiology of the soil will study bio-horizons, i.e. horizons still retaining the character of the vegetation which has formed them. The life of the soil, its history and relationship to the vegetation, its death and regeneration are the points which we will be looking for in bio-horizons of the soil.

The parent stratum, i.e. horizon D according to the nomenclature of morphologists-pedologists, does not lie in the depth of the soil profile but on its very surface. It is designated in soil science as A_0. Its character determines the character of further alterations in lower horizons, i.e. their physiology. Therefore, the most variagated subsoils may become the basis for the growth of most various forests. The physico-chemical condition of soil bio-horizons is in strict conformity with a definite type of vegetation which forms A_0.

The author has given a rough outline of a phytopedological classification in which the plant and soil are embodied in definite classes, each class having definite indexes of average normal conditions of correlation of the plant and soil.

Examples were given of the dynamics of phytopedological processes, and methods of determining the phase into which the said process has entered together with its trend were described.

In this case, however, the author has made use of but a few of the soil properties. Later, when physiological methods are applied to other soil properties our understanding of physiological processes in the soil will become clearer. Therefore, if questions of soil physiology will attract as much attention as do questions of soil morphology, the author, as a physiologist, will deem his purpose achieved.

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SOLUBILITY OF THE SOLID PHASE OF SOIL IN WATER

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Many investigators have studied the chemical action of water on minerals and rocks. Numerous laboratory investigations have been made on soil nutrients soluble in water. The action of water and salt solutions on diabase, porphyry, and silt loam was studied by Dietrich (2). Gedroiz (5, 6), changing the ratio between water and soil, investigated the solubility of soil compounds of saline and non-saline soils. The influence of the time of interaction and of the volume of solvent was studied by Zacharov (11). The same method of investigating the solubility of the soil combinations had been proposed formerly by Saidel (9). Vesterbergs (10), for the same purpose, applied three successive water extracts. Mitscherlich (8) studied the solubility of the soil in water saturated with CO_2.

The comparative study of soil solutions and water extracts may be considered as a kind of investigation on the solubility of soil compounds (1, 3, 4).

Water as a solvent of soil in comparison with other reagents has some advantages. In humid regions, water or dilute salt solutions are the medium of soil formation, plant growth, and microbiological activity. Therefore water applied as a solvent furthers the study of the processes of weathering, podzol formation, hydrolysis, and decomposition of the soil compounds in natural conditions. Besides, the influence of secondary accessory reactions from water extract is less than from other solvents.

The action of water on the soil consists in solution, hydrolysis, and decomposition of the soil compounds. In obtaining water extracts, the water acts first on easily soluble salts (nitrate, chloride, and sulfate of alkali metals). The salts of this type are usually dissolved in soil solutions and the water extracts them, even when the ratio of water to soil is small. The soil of humid regions does not contain many easily soluble salts. But under the influence of fertilization and tillage (fallow fields) the salt contents may increase to as much as 0.1 per cent. If the quantity of easily soluble salts in a soil is small and the soil does not contain carbonates and gypsum, the main action of the water is not solution but hydrolysis and decomposition of the composite soil compounds.

It is well known that minerals under the influence of water lose their bases and turn into SiO and R_2O_3 as final products of decomposition. The primary minerals (hornblende and others) probably being found in the soil, evidently

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react with water only in a colloidal state of dispersion. The theoretical principles of the reaction between the water and the colloid materials of the soil will be discussed further.

Our investigations were made in order to show the influence of various volumes of water on the solubility of the difficulty soluble soil compounds. For the investigations podzol and chernozem soils were taken at experiment stations of U. S. S. R. A brief description of these soils follows:

I. South chernozem of Kuban Experiment Station, 10 years uncropped, adsorbtion capacity 0.64 per cent Ca.
II. Chernozem of Kharkov district, virgin, adsorbtion capacity 0.77 per cent Ca.
III. Chernozem of Sumy Experiment Station (Ukraine), adsorbtion capacity 0.60 per cent Ca.
IV. Gray forest soil of Poltava, virgin, adsorbtion capacity 0.44 per cent Ca.
V. Podzol formed on marl of Viatka Experiment Station, uncropped, adsorbtion capacity 0.25 per cent Ca.
VI. Podzol of Engelgardt's Experiment Station, virgin, adsorbtion capacity 0.10 per cent Ca.
VII. Podzol of Experiment Field of the Agricultural Academy (Moscow), well-manured, uncropped, humus 1.5 per cent, adsorbtion capacity 0.17 per cent Ca.

These soils, taken from humid and subhumid regions, were free from carbonates and gypsum. The experiments were divided into two series:

1) ratio $\frac{\text{soil}}{\text{water}}$ ..... 1:1, 1:4, 1:10, 1:20, and 1:100

2) ratio $\frac{\text{soil}}{\text{water}}$ 1:4, 1:8, 1:16, 1:32, 1:64, 1:128

When the volume of water was small $\left(\text{ratio } \frac{\text{water}}{\text{soil}}\ 1:1\right)$ we obtained the liquid for the analysis by pressure in a little wine press. The water extracts were obtained by mechanical shaking for 10 minutes and filtering through an extra hard filter (602 Sch. und Schül). The first portion of the filtrate was discarded. The quantity of the electrolytes in the filtrate was determined by measuring the resistance on the Kohlrausch bridge at 18°C. The results of the first set of experiments are given in table 1, and of the second, in table 2.

These results prove that with the increasing of the masses of the solvent the quantity of soluble electrolytes increases though not proportionally.

It has been pointed out that the action of water on a non-saline soil consists mainly in first, solution of a small quantity of the easily soluble salts, and, second, in decomposition and hydrolysis of the soil colloidal material.

From a chemical point of view, the colloidal material of the soil—the adsorbing complex—may be considered as a combination of weak acids and strong bases. Michaelis (7) defines these compounds as saloid—heterapolaric chemical combinations insoluble in water and dissociated only on the surface. As a result of the dissociation on the surface of the soil particles there is formed a double electrical layer with numerous cations in the outer and one compara-

tively huge anion in the inner part of it. The saloids of the soil are more or less stable depending on the hydration of the cations.

TABLE 1

The quantity of the electrolytes in milliequivalent in kilogram absolutely dry soil

Values observed and calculated by equation $x = y\sqrt{V} + a$

SOILS	RATIO SOIL : WATER				
	1 1	1 4	1.10	1:20	1·100
Chernozem (I):					
Observed values	0 81	2.99	5.16	7 98	17.54
Calculated $y = 2.03$	0.81	2 84	5.19	7 85	19.07
Gray forest loam (IV):					
Observed values	1.17	2 13	3 86	...	11.65
Calculated $y = 1.12$	1.17	2 99	3.60	. .	11.27
Podzol (V):					
Observed values	2 25	2 63	. .	3 25	5 48
Calculated $y = 0.34$	2 25	2 59		3 43	5.33
Podzol (VI):					
Observed values	0.72	1 02	1.12	1 48	2 02
Calculated $y = 0.21$	0 72	0 94	1 18	1 46	2.63

TABLE 2

The quantity of electrolytes in milliequivalent in 1 kilogram dry soil

The values observed and calculated by equation $x = y\sqrt{V} + b$

SOILS	RATIO SOIL WATER					
	1 4	1 8	1 16	1 32	1.64	1 128
Chernozem (IV):						
Observed values	4 47	5 75	7.97	10.23	13 70	19 07
Calculated $y = 1.57$	4 47	5.76	7 61	10.21	13.89	19.38
Chernozem (II):						
Observed values	2.35	2 94	3 49		6.70	8 49
Calculated $y = 0.68$	2 35	2 91	3 71	.	6 43	8 81
Podzol (VII):						
Observed values	1.14	1 18	1.19	1.28	1 48	1 55
Calculated $y = 0.039$	1 14	1.17	1.22	1.28	1 37	1.51

When the quantity of easily soluble salts in the soil is small and the non-colloidal minerals dissolve slowly, the influence of water on the soil consists mainly in the reaction between the water and the colloidal complex (saloids). The hydrolysis of these saloids is similar to that on chemical components of

strong bases and weak acids. Ostwald proved that the molecular electrolytic conductivity of the slightly dissociated binary electrolytes increase as the square root of the volume of the solvent. The formula of dissociation can be figured as follows:

$$\frac{a^2}{(1-a)V} = K$$

where a is the dissociated part of the molecule and V the volume of the solution. As the dissociation of the saloid is very small, a may be regarded as 1, then $\frac{a^2}{V} = K$, from which $a = K\sqrt{V}$.

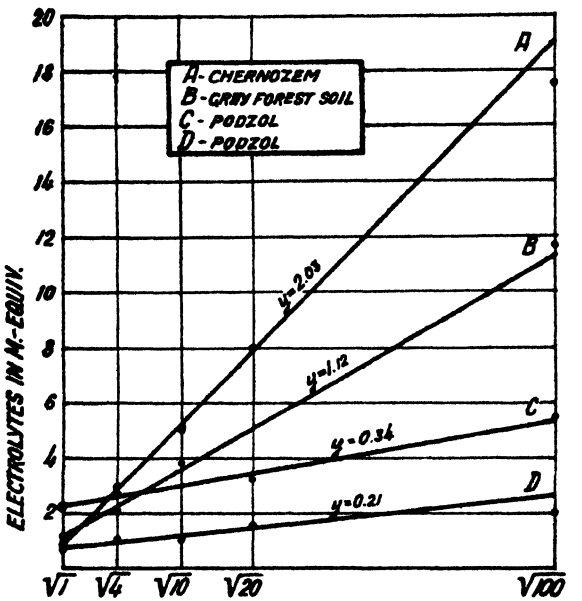

FIG. 1. SOLUBILITY OF THE SOIL COMPLEXES IN DIFFERENT SOIL TYPES

This formula, proposed by Ostwald only for cynare electrolytes was proved true also for the weak polybasic acids (fumaric, maleic, and others). Their dissociation proceeds according the equation $AcH_2 = A_cH + H$.

If we suppose that the dissociation of the fine colloidal material (saloid) runs similarly to the dissociation of the dissolved combinations, the interrelation between the quantity of the electrolytes extracted from the soil and the volume of the solution must follow the equation shown. The dissociation of many other non-colloidal combinations of the soil (compounds of strong acid and weak bases and the contrary) must follow the same rule.

The results of these investigations are represented by figures 1 and 2. The quantities of electrolytes are shown on the ordinate axis and on the abscissa—

the square roots of the ratio, soil to water. Evidently the connection between the quantity of electrolytes extracted from the soil and the volume of the water can be expressed by an irrational algebraic function $x = y\sqrt{V} + b$, where, x represents the quantity of soluble electrolytes in milliequivalents in 100 gm. absolutely dry soil, V the ratio between the volume of solvent and the weight of the soil, b the quantity (in milliequivalents) of the easily soluble salts extracted from the soil by small volume of water (ratio 1:1 or 1:4), y constant.

The calculated values x and y for each soil are represented in tables 1 and 2. The diviations of the observed and calculated values are within limits of the

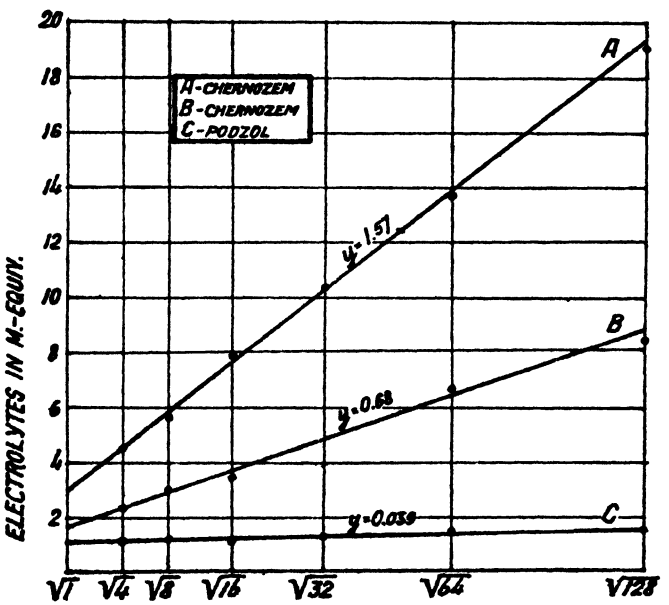

FIG 2. SOLUBILITY OF THE SOIL COMPLEXES IN DIFFERENT SOIL TYPES

errors of determinations. The values of the constants y for the chernozem are: 2.03 (Kuban), 1.54 (Sumy), 0.68 (Kharkow). The constant for the podzol soil fluctuates from 0.04 (well-manured soil) to 0.20 and 0.34, and the constant for the gray forest soil of Poltawa region = 1.12. In general the slope of the straight lines for the chernozem is more steep.

The position of the first point on the straight line is more or less accidental; it shows the quantity of easily soluble salts of the soil. The position of the other points will be determined mainly by the quantity of the soil colloidal substance, by the composition of the adsorbed bases, and partly by the solubility of the non-colloidal compounds of the soil. Hence the solubility in water of soil (free from carbonate and gypsum) is a definite property of the soil colloidal state.

If the solubility of the non-colloidal compounds is disregarded a close connection between the soil colloidal complex and the quantity of electrolytes in water extracts will be noted in the non-saline soil. We compared the quantity of electrolytes in water extracts (1:100) and the adsorption capacity of the soils in humid regions of U. S. S. R. The data are shown in table 3. Some connection between quantity of soluble electrolytes and adsorbed bases can be found in this table, but this relation is by no means quantitative.

The action of water on the adsorbed bases can be explained in another way—by the action of H from the dissolved CO_2 in water on the soil adsorbed bases.

TABLE 3

Electrolytes extracted by water (ratio 1:100) and adsorption capacity

SOILS	ADSORPTION CAPACITY IN M.E. IN 100 GM. ABSOLUTELY DRY SOIL	THE QUANTITY ELECTROLYTES IN M.E. IN 1 KGM. ABSOLUTELY DRY SOIL
Podzol VI	5.25	2 02
Podzol VII	8 60	4.57
Podzol V	12.3	5.48
Gray forest soil IV	22 1	11.65
Chernozem VIII	29.1	16 07
Chernozem I	34 1	17.53
271	17 0	11.0
189	20 25	7 8
193	22 9	10.9
210	27.2	16 2
195	31 2	16 3
240	33.1	16.1
214	39.8	16.6
188	41 9	17.6
220	40 4	21 7
224	45 4	23.1

In this case the quantity of soluble salts in the extracts will vary in proportion to the amount of CO_2 in the water.

In order to obtain the approximate method of determining the adsorbed bases we endeavored to investigate the electropermeability of $HgCl_2$ soil extracts. The dissociation constant of $0.01N$ $HgCl_2$ is

$$\frac{[Hg]\,[Cl]^2}{[HgCl_2]} = 1.5 \times 10^{-14}$$

The reaction between $HgCl_2$ and the adsorbed bases of the soil may be represented as follows:

$$(Ac)\ Ca + HgCl_2 \qquad (Ac)\ Hg + CaCl_2$$

Thus the electropermeability of these extracts is mainly determined by the quantity of replacable bases in the soil. Our negative data probably were a result of the decomposition of $HgCl_2$ and the solvation of the non-colloidal substances. The salts of aluminum, iron, phosphate, and carbonate must hydrolyze according to the rules given in the foregoing. This reaction proceeds simultaneously with hydrolysis of the soil-adsorbed complex. We compared the solubility of our soils with that of certain difficultly soluble mineral compounds. We studied the solubility: of $Ca_3(PO_4)_2$, of phosphate rock from Podolia, and of Khibin apatite.[2] The work itself as well as the method applied to the analysis was the same as for soils. The data of these analyses are given in table 4.

TABLE 4

Solubility of apatite, phosphate rock and tricalcium phosphate in milliequivalent per 100 gm.

	RATIO				
	1 4	1 16	1 32	1 64	1:128
Apatite (Khibin):					
Observed	1 85	2 62		3 84	4 92
Calculated $y = 0.34$ . .	1 85	2 53		3 89	5 04
Phosphate rock (Podzol).					
Observed	10 8	21 5	28 6	35.4	54 8
Calculated $y = 4.73$. ..	10 8	20 3	28 1	39 2	54.4

	RATIO				
	1 5	1 10	1 40	1 80	
Tricalcium phosphate:					
Observed	70 1	80 7	109.1	138 6	
Calculated $y = 11.1$ ...	70 1	80 4	115 4	144 5	

Under the given experimental conditions and within the range of the ratio of substance to water, from 1:10 to 1:100, rather close agreement of the calculated and observed values was obtained. The solubility of the phosphate from $Ca_3(PO_4)_2$ is much higher than that of the phosphate rock. The lowest solubility was shown by Khibin apatite. The values of the constant (y) successively were: for Ca_3PO_4 — 11.1, for Podolia phosphate rock 4.73, for Khibin apatite 0.34. The solubility of the relatively insoluble soil compounds was lower than that of phosphate rock. The constant for the chernozem soil solubility was higher than that of apatite. The value of y of podzol soil approached the constant of apatite solubility.

[2] The composition of Podolia phosphate rock: insoluble residue 3.72; P_2O_5 — 37.70; CaO — 48.78; CO_2 — 1.70; F — 2.5.

The composition of Khibin apatite insoluble residue — 8.4; P_2O_5 — 35.8 CaO — 47.43; F_2O_3 — 3.70; CO_2 — 0.0.

The apatite and phosphate rock were milled and passed through a 2-mm. sieve.

Therefore the expression $x = y\sqrt{V} + b$ is suitable for calculating the solubility of the phosphate minerals and other similar compounds. Thus by means of this equation, calculation of the solubility of soil phosphates and other compounds of the same type becomes possible. We worked out some of the data mentioned by Gedroiz (6) concerning the solubility of non-saline soil. The data on sodium carbonate and humus extracted from solonetz soil with subsequently increasing water volume are in full accordance with the values (shown in table 5) obtained by calculation. This agreement can be easily understood from a theoretical point of view, as sodium humus and carbonate are compounds of strong base and weak acid. The general amount of mineral residue extracted from the podzol soil by means of increasing water volume is similarly explained. The agreement was less close for the mineral residue of chernozem loam.

TABLE 5

Gedroiz's data

	RATIO									
	1·1	1 1.5	1:2	1 3	1 4	1 5	1·6	1 8	1·10	1.20
Solonetz:										
Observed.	99	...	166	231	280	325	366	432	490	780
Calculated	99	...	163	231	280	325	361	430	490	727
Podzol clay:										
Observed	189	204	213	240	268	280	294	344	370	400
Calculated.....	189	204	219	242 0	269.9	279.1	294	322	346 0	441

The solubility in water of individual soil compounds (phosphate and others) must follow the aforementioned formula. Investigations along this line are being continued.

The investigation of the electropermeability of water extracts from the soil may be interesting from another point of view. It is known that the basicity of acids and to some extent their structure can be determined by the electropermeability of salts of polybasic acids. Hence the study of the electropermeability of water extracts may be a means of investigating the nature of organic and mineral colloidal matter of the soil.

SUMMARY AND CONCLUSION

The solubility of chernozem and podzol soils (free from carbonate and gypsum) in water with different ratios of soil to water (from 1:1 to 1:128) has been studied. The quantity of soluble compounds has been determined by electrolytic conductivity of the water extracts. Two series of experiments have been set up:

ratio of soil to water. 1:1, 1:4, 1:10, 1:20, 1:100
ratio of soil to water..... 1:4, 1:8, 1:16, 1:32, 1:64, 1:128

For the tests the following soils were used: chernozem, podzol, and gray forest soil. The analysis of data reported in the paper leads to several conclusions. Between the volume of the solvent and the quantity of electrolytes exists a functional relation which can be expressed by the equation

$$x = y\sqrt{V} + b$$

where, x represents the quantity of soluble electrolytes in milliequivalents in 100 gm. absolutely dry soil, V the ratio between the volume of dissolvent and the weight of soil, b the quantity (in milliequivalents) of easily soluble salts extracted from the soil by a small volume of water, y constant.

The value of the constant (y) for chernozem fluctuated from 0.68 to 2.03, for podzol from 0.04 to 0.34. The observed and calculated data are shown in the tables 1 and 2.

The study of the solubility of phosphate minerals (apatite, phosphate rock) in water shows that the solution of these minerals to some extent follows the same rule. The constant (y) of the solubility of apatite was about the same as that of the podzol soil.

The data on sodium carbonate and humus extracted from solonetz soil with successively increasing water volume are in full agreement with the values obtained by calculation.

The investigation of the solubility of individual soil compounds in water is being continued.

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THE COLORIMETRIC DETERMINATION OF PHOSPHORUS IN CITRIC ACID EXTRACTS OF SOILS

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One of the important methods of studying soil fertility by chemical means is the citric acid extraction of Dyer (6, 7), with various modifications suitable to special local conditions. The determination of phosphoric acid in these extracts with reasonable accuracy has been a matter of considerable difficulty.

The precipitation of phosphorus as ammonium phosphomolybdate and the titration of the precipitate with sodium hydroxide and nitric acid have, at times, yielded extremely erratic results, even under the most carefully regulated conditions. For approximations of the probable reaction of soils toward phosphatic fertilization over large areas, the results of this method are doubtless of value in the main, but for accurate work on fertilizer experiments and for the determination of the fixing power of soils for phosphoric acid, a more accurate method is imperative.

Solution of the ammonium phosphomolybdate in ammonia, and double precipitation of phosphorus with magnesia mixture, although reliable for moderate or large amounts, is too long and tedious for routine soil analysis and is inaccurate in the case of citric acid extracts which contain small amounts of phosphorus.

Various colorimetric methods were therefore investigated, with the hope of applying one or more of them to the determination of phosphorus in citric acid extracts of soils; the object uppermost in mind was an increase in the accuracy of the results, together with a saving of time and labor, should this be possible, consistent with the reliability desired.

Successful results have been reported by Taylor and Miller (13), Denigès (5), Bell and Doisy (1), together with the modifications by Briggs (2) and Fiske and Subbarow (8). Parker and Fudge (12) studied these methods in relation to their merits for determining phosphorus in soil extracts and concluded that the method of Denigès is approximately five times as sensitive as that of Fiske and Subbarow. Improvements on the Denigès method were recently made by Truog and Meyer (15), who studied the effects of various interfering substances.

All of these methods, however, were intended primarily for use upon solutions relatively free from organic matter and such interfering cations as iron and titanium. Truog and

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Meyer (15) have stated that titanium and ferric iron interfere with the formation of the blue color, whereas ferrous iron does not; they recommend reducing the iron with cadmium in Jones reductors.

More recently, Chapman (4), using the Truog and Meyer method, has shown that the presence of even ferrous iron may be objectionable under certain circumstances; a method for overcoming this difficulty is proposed, but no mention of the effect of titanium is made. The work of Truog and Meyer indicates that concentrations of 20 p.p.m. of this cation interfere seriously.

In the case of colorimetric methods specially intended for the determination of phosphorus in citric acid extracts, extreme rapidity appears to be the object principally sought by investigators. Němec, Lanîk, and Koppová (11) treat 5 ml. of the extract with sulfuric acid and potassium permanganate and then reduce the excess permanganate with hydrogen peroxide. However, no comment is forthcoming about the effect of iron and titanium, and experiments on extracts of Hawaiian soils have shown that they interfere to such a marked extent as to render the procedure unavailable for use. Moreover, potassium permanganate of the regular C.P. grade carries so much phosphorus that the blank is of the same order of magnitude as the amount of phosphorus in the sample.

Similar objections are valid for the method whereby Zinzadze (17, 18) prepares the solution for analysis; iron was found to interfere.

Warren and Pugh (16) recognized the influence of ferric iron upon the development of the blue color, stating that in certain instances the greenish yellow tint became so pronounced that it was impossible to make comparisons. They developed a procedure involving the destruction of organic matter with sodium permanganate and the removal of iron as the ferrocyanide. Here again, however, the amount of phosphorus present in the reagents is of the same order of magnitude as that in the sample, and inaccurate results are certain to follow in the case of soils low in P_2O_5. Further, the ferrocyanide precipitate is difficult to filter, because of its colloidal condition.

EXPERIMENTAL

Precipitation of iron and titanium by sodium hydroxide was attempted, but it was found impossible to remove all the phosphorus from the precipitate even by double precipitation and repeated washing with hot hydroxide solution containing sulfate. The possibility suggested itself that incomplete recovery might be apparent rather than actual, because of the presence of some substance interfering with the development of the color in the filtrate. But the only substance known to interfere, which might be present in more than negligible amounts in the filtrate from sodium hydroxide precipitation, is quinquevalent vanadium. A determination of the amount of vanadium was therefore made; 0.76 p.p.m. of V_2O_5 were found and tests showed that this amount had no appreciable effect on the formation of the blue color. Chapman's work indicates that many times this amount of vanadium may be present without deleterious influence. It seems, therefore, that incomplete recovery of phosphorus from sodium hydroxide treatment is due to its being held in the precipitate.

Thornton (14) has stated that cupferron, the ammonium salt of nitrosophenylhydroxylamine, is the only known precipitant for titanium that does not coprecipitate orthophosphoric acid. A quantity of cupferron was prepared according to the directions given by Thornton (14, p. 224), and iron, titanium,

vanadium and the like were precipitated directly from the citric acid extracts in the presence of hydrochloric or sulfuric acid. It was found, however, that the excess cupferron was very difficult to destroy. Fusion with magnesium nitrate was unsatisfactory, as was treatment with aqua regia. Gentle ignition in the muffle rendered the residue extremely difficult to redissolve, and treatment with potassium permanganate was inadvisable because of the amount of phosphorus which that reagent carries. Repeated evaporation with nitric and sulfuric acids was successful in destroying the cupferron, but not unless the solution was heated to a temperature at which copious evolution of SO_3 fumes resulted, and under these conditions losses of phosphoric acid occurred by evaporation. In another series of experiments the cupferron was partially destroyed by evaporation with aqua regia and the phosphorus separated by precipitation with ammonia after the addition of aluminum sulfate. This procedure gave low results at times, however, because of incomplete precipitation of the phosphorus.

Electrolytic separation of iron using a mercury cathode

Experiments were then devised upon the electrolytic separation of iron from the solutions, prior to developing the color, using a mercury cathode in which to collect the deposited iron. A very satisfactory separation was obtained, but the titanium remained in solution. Such a neat and convenient removal of the iron, which is the worst interfering cation, resulted, however, that a colorimetric method which was not influenced by titanium was sought.

The method of Truog and Meyer had to be ruled out immediately, since as little as 20 parts of titanium per million result in serious difficulty, and many Hawaiian soils give citric acid extracts in which the concentration of titanium greatly exceeds this amount. Further, the color developed by this method is not stable, increasing in intensity during the first 5 minutes and then gradually fading after 10 or 15 minutes. This instability of color appears to be characteristic of all molybdenum blue methods for phosphorus which call for an excess of reducing agent in the solution.

In the method of Zinzadze (18) no excess reducing agent is present and in consequence the color developed is very stable, remaining undiminished in intensity over a period of days. With this advantage in its favor, the Zinzadze method was definitely adopted when it was found that titanium had little or no influence upon the development of the blue color.

Influence of titanium

In this experiment, a series of solutions containing 2.0 parts of P_2O_5 per million were used, to which varying amounts of titanium had been added. The results of duplicate determinations by the Zinzadze method are shown in table 1.

These results show that titanium may be present in concentrations up to 150 p.p.m. without influence. Although the colors of the solutions con-

taining 200 and 250 p.p.m. were not compared in the colorimeter with standards because of their cloudiness, a rough comparison showed that they were somewhat less intense, because of precipitation of titanium phosphate. It may therefore be concluded that titanium is not harmful unless it reaches a concentration high enough to precipitate some of the phosphate, under which circumstances it will reveal its presence so that the analyst will be forewarned.

Departure from Beer's law in pure solutions

Since it was desired to compare the colors of unknown solutions with standards in the Klett colorimeter, an experiment was conducted to determine how closely Beer's law was followed; this law states that the intensity of color is

TABLE 1

Effect of titanium upon color development

P_2O_5 PRESENT	Ti PRESENT	P_2O_5 FOUND BY ZINZADZE METHOD
p.p.m.	*p.p.m.*	*p.p.m.*
2.0	10	2.05
2.0	10	2.00
2.0	25	2.02
2.0	25	2.04
2.0	50	1.96
2.0	50	1.97
2.0	100	1.93
2 0	100	1.97
2.0	150	2.00
2.0	150	2 01
2.0	200	*
2.0	200	*
2.0	250	*
2.0	250	*

* Intensities not read in the colorimeter; titanium phosphate was precipitated which rendered the solutions cloudy.

proportional to the concentration. Accordingly a series of standards was made up from pure potassium phosphate solutions and the colors compared with that of a solution containing 2.5 p.p.m. of P_2O_5. The average results of duplicate determinations are shown in table 2.

The relation of these results to the theoretical values is shown in figure 1, where milligrams of P_2O_5 present per hundred ml. of solution are plotted against the milligrams per hundred ml. found by colorimetry. The heavy line represents the data, while the light 45° line is the theoretical curve. It is evident that in pure potassium phosphate solutions the deviation from Beer's law is very slight over this range. A very good approximation may be obtained by assuming the theoretical ratio as the basis of calculations, while for the most exact work, the true values may be read from the experimental curve. Since

the standard solution used for comparing the color contained 0.25 mgm. of P_2O_5 per 100 ml., the experimental curve should cross the 45° line at this point, namely, 0.25; the fact that it does not, indicates a small constant error, tending to make the results a trifle high, independent of the deviation from Beer's law.

TABLE 2

Departure from Beer's law in pure phosphate solutions

P_2O_5 PRESENT	P_2O_5 FOUND	P_2O_5 PRESENT	P_2O_5 FOUND
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
0.50	0 58	3 00	3 00
0.60	0 70	3 20	3 32
0 80	0 91	3 40	3 51
1 00	1 03	3 60	3 75
1.20	1 29	3 80	3 90
1.40	1 48	4 00	4 11
1.60	1 68	4 20	4 27
1.80	1 85	4 40	4 39
2 00	2 10	4 60	4 63
2.20	2 22	4 80	4 88
2 40	2 44	5 00	5 02
2 60	2 57	5 20	5 20
2 80	2 86	5 40	5.42

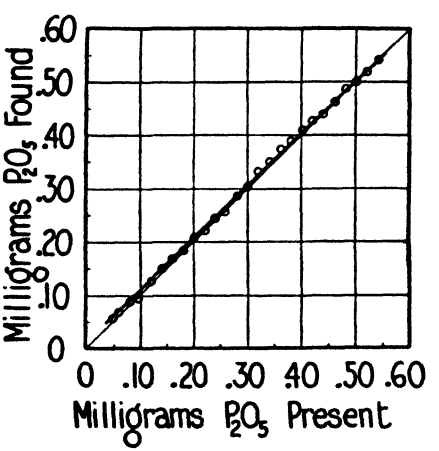

FIG. 1. DEVIATION FROM BEER'S LAW IN PURE POTASSIUM PHOSPHATE SOLUTIONS

Deviation from Beer's law in citric acid extracts

Although the deviation from Beer's law is slight in pure phosphate solutions, the conclusion is not justified that such will be the case in the solutions actually used for analysis and which contain a great many other salts. A citric acid extract which had been prepared for analysis as described in the following was therefore used to determine how closely Beer's law was followed in such a

solution. The procedure was similar to that used for pure phosphate solutions. The standard contained 0.30 mgm. of P_2O_5 per 100 ml., and aliquots of varying size were taken from the unknown. Since the concentration of P_2O_5 in the extract was not known, it was necessary to determine this value in such a way as to avoid error due to deviation from Beer's law. This could have been done by a method of trial and error, in which a series of aliquots of the unknown solution would have been taken until an aliquot volume was found which

TABLE 3

Departure from Beer's law in a citric acid extract

ALIQUOT OF UNKNOWN TAKEN	P_2O_5 PRESENT PER 100 ML.	P_2O_5 FOUND PER 100 ML.
ml.	*mgm.*	*mgm.*
1	0 071	0 078
2	0 142	0 141
3	0 213	0 199
4	0 284	0.288
5	0 355	0 351
10	0 711	0.670
15	1.066	0 944
20	1.422	1.12

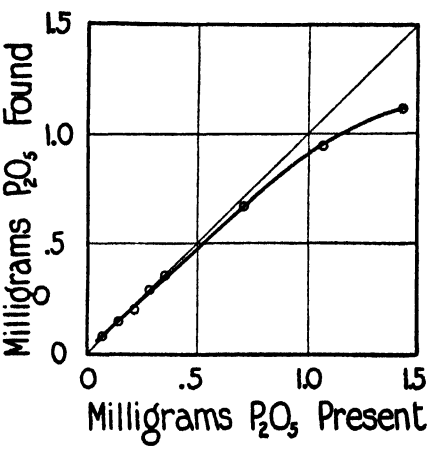

FIG. 2. DEVIATION FROM BEER'S LAW IN A CITRIC ACID EXTRACT

exactly matched the color intensity of the standard and which, of course, would have given a value of unity for the ratio between standard and unknown solution in the colorimeter. It was more convenient, however, to plot the volumes of the various aliquots actually taken in the experiment, against the values of the corresponding colorimeter ratios found and, having drawn the curve representing the data, to read therefrom the volume of the unknown necessary to give a colorimeter ratio of 1. The concentration of the unknown solution

was then calculated from the aliquot volume so determined and the known concentration of the standard. From this value, the actual concentrations of the various solutions were calculated from the volumes of the aliquot portions taken. The resulting data are shown in table 3.

The results are plotted in figure 2. The range of ratios of standard to unknown is somewhat wider than in figure 1, but even so the departure from Beer's law is considerably greater than in the pure solutions; it therefore behooves the analyst to use a standard which is very close to the unknown in color intensity if accuracy is desired.

TABLE 4

Effect of dilution upon color intensity

P_2O_5 PRESENT PER 100 ML.	P_2O_5 INDICATED PER 100 ML.	STRONG STANDARD DILUTED TO 100 ML. BEFORE READING
mgm.	*mgm.*	*ml.*
0 05	0 037	5
0 10	0 084	10
0 15	0.133	15
0.20	0 180	20
0 25	0 225	25
0.30	0.265	30
0 35	0.317	35
0 40	0 375	40
0 45	0.414	45
0.50	0 465	50
0 60	0 561	60
0 70	0 668	70
0 80	0 779	80
0 90	0 892	90

Influence of dilution upon color

Since the color developed by the Zinzadze method is quite stable, as opposed to procedures where an excess of reducing agent is present, the obvious solution to this difficulty is to prepare a wide range of semi-permanent standards; Zinzadze claims that standard solutions retain their full intensity for periods of 7 to 10 days. The writer, however, cannot concur in this matter, having found diminutions of 10 per cent in color intensity after 3 days' standing in the dark.

The inconvenience of making up a large number of standards very frequently, or of taking a second aliquot of the unknown in order to bring it near a single standard, may be avoided by preparing a strong standard and diluting with water till the intensity is close to that of the unknown. Standard color solutions prepared by dilution are not comparable with standards made up in the regular manner, but their values may be determined from a curve. A comparison of diluted standards containing the same amount of P_2O_5 as

standards prepared in the regular manner is shown in table 4. In the left column of the table are shown the actual number of milligram of P_2O_5 present in 100 ml., and the middle column shows the number of milligrams of P_2O_5 per 100 ml. which diluted standards of the same concentrations appeared to contain.

The data of table 4 are plotted in figure 3 and this curve is used to determine the color value of any diluted standard which is made up from the strong one containing 1.00 mgm. of P_2O_5 per 100 ml.

ANALYTICAL

Preparation of citric acid extract for analysis

Apparatus and reagents.—The cell used for the electrolytic separation of iron is a simple modification of the Cain (3) electrolytic cell; it consists of a straight

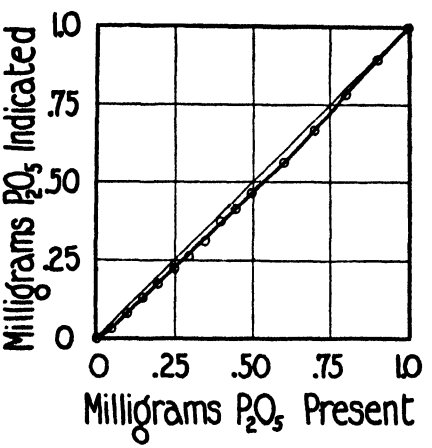

FIG. 3. EFFECT OF DILUTION WITH WATER UPON THE COLOR OF A STANDARD SOLUTION

soil extraction tube having a capacity of about 150 ml. The small glass tube at the bottom of the extraction tube is provided with a short length of rubber tubing and a screw clamp for drawing off the mercury when the electrolysis is completed. The electrodes are supported by a stopper at the top of the extraction tube, the anode being a platinum spiral and the lead to the cathode a short piece of platinum wire sealed in glass, which dips into the mercury at the bottom of the cell, as shown in figure 4. A battery of 20 such cells can be made up simply and cheaply and they are quite satisfactory, although cells provided with glass stop cocks would be considerably more convenient. Cells with rotating anodes, permitting the use of much higher amperages would doubtless be ideal for this purpose, since the time of electrolysis could be enormously reduced.

The mercury used for the cathodes should be purified by washing with hydrochloric acid and subsequently with water before distilling. A small automatic

mercury still (10) may be constructed by anyone with elementary skill in glass blowing and requires no attention other than maintaining the charge of impure mercury and collecting the distillate.

Procedure.—To 100 ml. of citric acid extract, add 50 ml. of concentrated nitric acid, 15 ml. of concentrated hydrochloric acid, and 10 ml. of 20 per cent sulfuric acid free from phosphorus and arsenic. Evaporate slowly till fumes of SO_3 are evolved, being careful not to prolong the evolution of fumes unduly, nor to elevate the temperature excessively, lest phosphoric acid be lost by evaporation. Take up in hot water and boil to insure resolution of any precipitate other than silica which may have formed. Filter into an electrolytic cell provided with a mercury cathode and a platinum spiral anode and electrolyze under a current density at the cathode of 0.03 to 0.05 amperes per

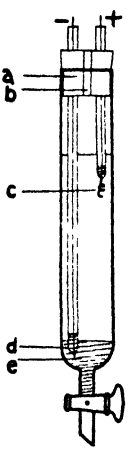

FIG. 4. ELECTROLYTIC CELL FOR SEPARATION OF IRON

a, stopper; *b*, gas vent; *c*, platinum spiral anode; *d*, platinum lead to cathode; *e*, mercury cathode.

square centimeter and a pressure of 6 to 8 volts. After 16 hours, withdraw the mercury from the bottom of the cell and filter the solution into a beaker. Add a few drops of 0.25 per cent hydrogen peroxide in order to reduce any permanganate which may have formed and concentrate to half the original volume in order to insure that the excess hydrogen peroxide, as well as the dissolved hydrogen and oxygen from the electrolysis, are driven off. Transfer to 100-ml. volumetric flasks, take an appropriate aliquot and develop the color by the method of Zinzadze.

Color development by the method of Zinzadze

Reagents.—Molybdenum blue reagent: Heat 50 ml. of phosphorus- and arsenic-free concentrated sulfuric acid to boiling, add 3 gm. of C.P. powdered molybdic acid and boil for 10 or 15 minutes until solution is complete. After

cooling, pour into 50 ml. of water and while still hot add 0.15 gm. of highest purity powdered molybdenum metal as reducing agent; finally, boil the solution for 3 to 5 minutes. After settling 10 to 15 minutes, the blue liquid is decanted from the undissolved metal, cooled, and made up to a volume of 100 ml. It is essential that the reducing power of the reagent be carefully standardized; 2.5 ml. of the solution should bleach exactly 0.2 ml. of normal potassium permanganate. If the reagent is found too weak, it is boiled again with metallic molybdenum, or mixed with the calculated quantity of stronger standardized solution; if it is too strong, it is diluted with the calculated quantity of unreduced 50 per cent sulfuric acid—molybdic acid solution.

Standard phosphate solution: Weigh out 0.1917 gm. of pure recrystallized potassium phosphate (KH_2PO_4) and dissolve in 1 liter. Take a 50-ml. aliquot of this solution and dilute to 500 ml. The later solution contains 0.010 mgm. of P_2O_5 per ml. Transfer 25 ml. of the dilute standard to a 100-ml. volumetric flask, dilute to 90 ml., add 1.4 ml. of molybdenum blue reagent, heat on the steam bath for 30 minutes, cool, and make up to exactly 100 ml. This gives a color standard of convenient strength for use in the colorimeter, but the concentration may be varied to suit individual taste.

The strong solution which was used in determining the dilution curve of figure 3 contained 0.010 mgm. of P_2O_5 per ml.

Determination.—From the solutions of citric acid extract as prepared for analysis, transfer aliquots of from 5 to 50 ml. to 100-ml. volumetric flasks and neutralize to the yellow end point of alpha-dinitrophenol indicator with 10 per cent ammonia solution. Dilute the solutions to 90 ml., add 1.4 ml. of molybdenum blue reagent, heat for 30 minutes on the steam bath, cool, and make up to exactly 100 ml. Employ a sensitive colorimeter for comparison with the standard solution. The ratio between strength of standard and of unknown should be very close to 1 if the highest accuracy is desired; if the standard has been prepared from a stronger solution, in which the color is already developed, by dilution with water as described in the foregoing, a curve similar to figure 3 should be used for correcting the value of the standard used.

Results obtained by employing the method described.—Table 5 shows the degree of reproducibility which may be expected from duplicate determinations. These analyses were made in a regular routine manner without the observance of any elaborate precautions and are representative of some hundreds of duplications obtained at different times and by different analysts, using the method described.

Although good checks are obtained, it still remains to be shown that no proportional errors of any appreciable magnitude are present. For this purpose, a citric acid soil extract was selected for experiment, and varying amounts of P_2O_5 were added to equal aliquots of the extract, in order to learn whether added P_2O_5 might be completely recovered from such solutions.

In table 6, column 1 shows the amounts of P_2O_5 added to 100-ml. aliquots of the extract, and column 3, the amounts found by colorimetric analysis.

In column 2 the amounts calculated for each aliquot from the amounts found in the solutions which received no additions of P_2O_5 are shown, and the per cent deviations of the experimental from the calculated values appear in column 4.

The average deviation is 3.8 per cent and the average recovery, 102.6 per cent. The indicated error in recovery, therefore, is within the experimental

TABLE 5

P_2O_5 soluble in 1 per cent citric acid

PER CENT P_2O_5		PER CENT P_2O_5	
I	II	I	II
0 0345	0 0348	0 0320	0.0320
0 0178	0 0179	0 0369	0 0366
0 0215	0 0248	0 0384	0 0389
0 0386	0 0382	0 0400	0 0403
0 0380	0 0368	0 0417	0 0417
0 0500	0 0540	0 0116	0 0113
0 0352	0 0350	0 0123	0 0119
0 0470	0 0415	0 0227	0 0221
0 0230	0 0220	0 0238	0 0239
0 0240	0.0240	0 0258	0 0254
0 0258	0 0252	0 0278	0 0268

TABLE 6

Recovery of added P_2O_5 from a citric acid extract

P_2O_5 ADDED	P_2O_5 CALCULATED FROM AMOUNT IN UNTREATED EXTRACT	P_2O_5 FOUND	DEVIATION
mgm.	*mgm.*	*mgm*	*per cent*
0	4 6	4 2	.
0	....	4 9	...
5	9 6	10.1	4 9
10	14 6	14.9	2.0
15	19 6	19 4	1 0
20	24 6	26 4	6 8
25	29 6	31 5	6 0
35	39.6	41.5	2 2
40	44 6	46 3	3 7

error of the method, and evidently no error proportional to the amount of P_2O_5 present appears.

CONCLUSIONS

The stated amounts of hydrochloric and nitric acids are sufficient to destroy all citric acid, as well as organic matter from the soil, in extracts of Hawaiian soils. Soils carrying more organic matter may require further additions.

According to Zinzadze, as much as 700 p.p.m. of SiO_2 has no influence upon the color development and it has been found that separation of most of the silica by dehydration with hot fuming sulfuric acid is a great deal more satisfactory than evaporation to dryness with hydrochloric acid and baking; enough of the silica is eliminated to prevent interference. In the latter method, the large amount of titanium present in Hawaiian soils precipitates a considerable amount of phosphorus which will not redissolve in hydrochloric acid, and for accurate work the residue must be fuzed with sodium carbonate in order to recover the phosphorus.

At the close of the evaporation the sulfuric acid must not be allowed to fume vigorously or at a high temperature, since there is danger of loss of phosphoric acid.

The mercury should be drained from the cells during electrolysis, or immediately thereafter, to prevent resolution of iron.

For the best results, the color should be developed in the standards coincident with developing the color in the unknown solutions.

Special care should be exercised to avoid contamination from foreign sources, such as reagents and glassware. Blanks should be run frequently, preferably with the addition of a known amount of standard phosphate solution just before developing the color, in order to reduce errors due to deviations from Beer's law at the low concentration of the blank.

With the observance of these precautions it should be possible to obtain duplicate analyses differing by no more than 5 per cent at the outside.

SUMMARY

A modification of the Zinzadze colorimetric method for determining phosphorus in citric acid extracts of soils high in iron and titanium has been described.

A method of separating iron from the solutions has been outlined.

It has been shown that as much as 150 parts of titanium per million does not interfere.

The departure from Beer's law in citric acid extracts as contrasted with such departure in pure phosphate solutions has been investigated and reported.

The influence of dilution of the blue color developed by the Zinzadze method has been shown.

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THE RELATION OF CALICHE TO DESERT PLANTS

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Over extended areas in the southwestern United States the soil is impregnated with faintly stratified layers of calcareous hardpan of varying thickness, or filled with hard nodules and amorphous masses of highly calcareous material. In certain localities the nodules have become cemented together into large masses with many lacunae. Associated with these deposits is a finely divided material which contains a small percentage of normal soil constituents and such a high percentage of salts as to be white or nearly white in color. The common type of calcareous hardpan is known in the southwestern states by its Spanish name *caliche*, a word also used in Chile for the analogous deposits of sodium nitrate.

Many types of hardpan have been described and differences have been noted between their location in the soil, their texture, and their composition in the various regions in which they occur. Also, there is general agreement that the manner of formation is not always the same. The position of the material is always closely parallel to the soil surface. The commonest types are found either just below the customary level of tillage or else at the level to which the deepest roots penetrate. Weaver and Crist (8) have described the hardpan of the Great Plains, which lies at a depth of 15 to 36 inches, has a thickness of 8 to 18 inches, and is readily penetrated by roots. This type is stated to disappear when the soil is moist, and its existence is evidently of minor importance in relation to moisture movements in the soil and to root development. Nothing has been done toward a comparative study of the different types of hardpan and little has been written that helps in forming a picture of their geographic distribution. The rather distinctive type which is designated as caliche is known to be of wide occurrence in Arizona, New Mexico, and Sonora, and very similar deposits have been found in adjacent regions.

The observations of the writers have been made chiefly within a 200-mile radius of Tucson, Arizona, and the material of caliche for experimental purposes has been taken within 30 miles of Tucson. In this region caliche is abundant in the soils of outwash plains, or bajadas. The heaviest deposits are found in the lower parts of those bajadas which have been built up solely or largely by outwash from volcanic mountains. Caliche is also found in the soils of limestone and volcanic hills, covering the submerged rock surfaces or filling crevices. In general it is absent from purely granitic soils, from sand, from

the heavy clay, or "adobe," of alluvial flood-plains, and the similar but somewhat coarser alluvial soil of poorly drained basins, or "playas."

The uppermost layers of caliche may be found very close to the surface or at depths as great as 2 or 3 m. They are exposed to view or found very near the surface only in places that have been subjected to recent erosion. The surface of the caliche is undulating and relatively smooth. The thickness of the layers ranges from a few millimeters to 40 cm., but is most commonly between 2 and 5 cm. The lateral extent of the layers is extremely variable, and the continuity of the surface is frequently broken. Approximately the upper half of the surface layer is so hard that it grinds slowly under a file or emery wheel, is very homogeneous in structure, shows a distinct horizontal lamination, and is free of stones and soil particles (pl. 1, fig. 1). The lower portion is somewhat softer, without visible structure, and filled with a greater or less number of stones of various sizes. Beneath the caliche may be found a normal soil, or more often a soil so highly impregnated with salts as to be nearly white, containing pebbles or nodules of material closely similar to the layers of caliche. With increasing depth, successive layers of caliche are found, varying in thickness and frequency, sometimes similar to the surface layer or in other places somewhat softer and less sharply defined. In the larger valleys of southern Arizona, which have been filled with detrital material to great depths, the recurring layers have been found in numerous well digging operations. Under the eastern part of Tucson they have been found in close succession to a depth of 30 m. Meinzer and Kelton (5) have reported cemented layers at a depth of 125 m. in two wells sunk in valley fill at Douglas, Arizona, and definite caliche layers extending for 90 m. to a depth of 230 m. in a well at San Simon, Arizona.

The composition of caliche varies from place to place. Analyses made by the Arizona Agricultural Experiment Station show a content of 55 to 90 per cent calcium carbonate, which is always the predominant constituent. Magnesium carbonate varies from 2 to 5 per cent, and iron, sodium, potassium, and phosphorus are present in small quantities. In some samples calcium silicate and aluminum silicate are present in quantities varying from 10 to 15 per cent. These compounds are generally more abundant in the surface layers and are responsible for their hardness.

Several hypotheses have been advanced in regard to the formation of caliche. All of them take into account the abundance of calcium and the other constituents in the ground water, soils, and common limestone and volcanic rocks of the region. All of them emphasize the important rôle of active evaporation in causing the accumulation of calcareous residues in the soil. Blake (1) laid emphasis on the importance of the upward movement of ground water into levels at which sub-surface evaporation would take place. Lee (4) described localities in the Salt River Valley where caliche was absent from the uppermost 4.5 m. of soil but occurred in the upper layers of the water-bearing gravels which lay just beneath the soil. Forbes (3; 4, p. 110) has attributed the forma-

tion to the penetration of rain water, which dissolves the available salts during its descent and finally precipitates them at the lowest level to which it penetrates. In a recent publication Breazeale and Smith (2) have reviewed all of the hypotheses and their evidences, and have concluded that different groups of factors have been concerned in the formation of the superficial caliche of the bajadas, the deep-seated caliche described by Lee, and the deposits formed on and between rocks on hillsides and mountain slopes. They have also emphasized the importance of plants in removing soil water and causing precipitation at the level of absorbing roots. Breazeale and Smith have accounted for the formation of caliche in bajada soils by reviving the wholly untenable hypothesis of its formation under water in shallow lakes and pools.

The occurrence of successive layers of caliche to a considerable depth is to be accounted for by the gradual upbuilding of the bajadas through deposition of material from the adjacent mountains. Such layers must be regarded as due to descending surface water, since capillarity could not be invoked to raise the ground water, lying from 15 to 25 m. below, if indeed present at all. In soils with a high ground water table or a continuously high water content, it is on the surface that the ascending water causes deposition of salts to take place —the well known "alkali spots" of irrigated desert land.

As maintained by Forbes, the type of caliche found near the surface on the nearly level bajadas of southern Arizona may be regarded as due to descending surface water, limited by climatic conditions in the depth to which it penetrates the soil, and aided by plant roots or soil organisms in the liberation and deposition of its salts. Evidence for the influence of arid conditions on the descending soil water may be had in the rather infrequent occurrence of caliche in the form of small stalactites (pl. 1, fig. 2). These are found where stones or large bodies of caliche are underlaid by small cavities in the ground. The infrequency of the stalactites is solely due to the rare occurrence of such cavities. The hypothesis of Forbes explains all of the observed facts and conditions but still leaves it difficult to account for the absence of caliche from the adobe clay of flood-plains and the similar soil of playas.

INFLUENCE OF CALICHE ON WATER RELATIONS OF THE SOIL

To the student of vegetation, the origin and mode of formation of caliche are not so important as its influence on the physical and chemical properties of the soil and on the development of the root system. The object of the present work has been to investigate some of the physical characteristics of caliche with reference to the movements and availability of water in the soil, and to determine the influence exerted on the growth of a common desert shrub by varying quantities of the material of which caliche is composed.

The degree of insolubility of caliche in water is indicated by the high percentage of calcium carbonate which it contains and by the silicates which are present in the harder types. The presence in soil water of dissolved carbon dioxide, derived from the atmosphere or from plant roots, greatly increases the solubility of caliche under natural conditions.

The structure of the stratified surface layers of caliche is of such density as to provide a very small volume of air space replacable by water on wetting. Seven pieces of air-dried caliche weighing between 100 and 200 gm. were immersed in water for 48 hours. They were then removed from the water, blotted, and allowed to dry on the surface. The percentages of water which they contained, based on the dry weight, were 4.7, 6.5, 3.2, 3.7, 3.0, 4.8, 5.4. These percentages indicate a very low water content as compared with soils. It is evident that caliche is of no importance as a reservoir of moisture, and that it does not have a great enough water capacity to remove any considerable quantity of water from the adjacent soil by capillarity and to hold it where it is not available to plants. The soft type of caliche is much more porous, and similar determinations of its maximum water content have shown it to vary from 12.9 to 17.3 per cent, amounts which are comparable to those found in soils.

When the edge of a dry piece of hard caliche is placed in water it is possible for a short time to observe the wetting by the darkening of the color. The advancing edge of the moist zone soon becomes indistinct, however, even when a dye is used, so that it is not possible to measure easily the time required for the wetting of the entire piece. Neither is the advance of water on the surface an accurate indication of its advance in the interior.

The influence of a single layer of hard caliche on the capillary rise of water in a column of soil was determined by the following experiment. Two glass cylinders were selected, 6.5 cm. in diameter and 15 cm. high. A piece of caliche averaging 1 cm. in thickness was turned down on an emery wheel to the inside diameter of the cylinders and was cemented into one of them 8 cm. from the bottom. A fine clay soil in air-dry condition was then tamped into the cylinder above and below the caliche and the second cylinder was filled in the same manner with soil only. The two cylinders were then placed in a dish with water 2 cm. deep. The experiment was started at noon on May 19. By 2 p.m. of the same day water had reached the top of the control cylinder and the bottom of the caliche layer. At 2 p.m. on May 20, water had penetrated the caliche on one side of the cylinder to a distance of 7 mm. At 4 p.m. on May 21 it had penetrated the entire layer, or at least reached the entire circumference of the cylinder, to an average distance of 1.8 cm. The slow rise of water was measured on successive days and had reached 4.5 cm. above the layer on May 27. The free rise of water to the 8-cm. level in both cylinders required 1 hr. 45 min., whereas the rise above the layer of caliche was 4.5 cm. in 7 days. A repetition of the experiment with another piece of caliche 17 mm. thick and apparently of a somewhat more porous character gave similar results, with movement about 10 per cent greater than in the first case.

In order to test the downward movement of water through caliche an experiment was performed with a cylinder arranged precisely as just described, with room left at the top for placing water to a depth of 1 cm. As water penetrated the soil the amount was renewed to the original level. On the seventh

day the water was poured off the surface, 2 cm. of soil were removed from the top and discarded, and determinations were made of the moisture content of the soil above the caliche layer and of that below it. On the basis of dry weight, the percentages were respectively 34.8 and 18.8. These experiments indicate that even a thin layer of caliche constitutes a serious obstacle to the movement of water in the amounts and at the rates which are possible in free soil.

Edith B. Shreve (7) has shown that a caliche layer may act as a semi-permeable membrane, and it is possible that relatively thin layers with adjacent bodies of soil may play an important part in the movement of water under conditions where soil solutions of different osmotic value exist on the two sides of the caliche. Such movements might be great enough in critical seasons to be of importance to plants.

When the soft type of caliche is reduced to a coarse powder it possesses a mechanical composition close to that of a fine alluvial clay. A comparison of the capillary rise of water in this material and in "adobe" clay was made in the following manner. Tubes 1 cm. in diameter were filled (*a*) with adobe clay, (*b*) with powdered caliche, (*c*) with alternating layers of the two, 10 cm. thick, (*d*) with alternating layers of clay 5 cm. thick and of powdered caliche 1 cm. thick. The capillary rise of water in the four tubes proceeded at nearly the same rate, and in 12 hours reached levels varying from 29 to 30 cm. above the level from which water was drawn. It appears, therefore, that a wide difference exists between the influence exerted on the movement of soil water by layers of hard caliche and by the soft masses which are frequent at lower levels and are easily reduced to the form used in this experiment. Considerable importance attaches to the rate at which water is lost by evaporation from the smooth surface of hard caliche, both in the rare cases in which it is exposed at the soil surface and in the commoner cases in which it is overlaid by a thin and very dry body of soil.

In order to give a basis for comparison of the evaporation from caliche and from the surface of the structurally analogous porous cup atmometer the following experiment was performed. An atmometer was mounted on a glass tube with attached burette. A funnel 9 cm. in diameter was mounted in the same manner. A piece of hard caliche 3 cm. thick was turned so as to fit closely into the funnel, with its upper surface flush with the edge, and was then cemented into place. Water was introduced and a burette attached.

The two evaporating surfaces were placed side by side in the laboratory and the losses read from the burettes. The results, shown in table 1, indicate that the loss from comparable areas was about one-fifth as great from the caliche as from the atmometer. An effort to obtain readings at a higher rate of evaporation resulted in the breaking of the capillary columns of water in the caliche and the appearance of bubbles beneath it.

A further experiment was carried out to determine the rate of evaporation under more natural conditions, using thinner layers of caliche to which water

was supplied by a moist soil. Six pieces of caliche, varying from 5 mm. to 3 cm. in thickness, were turned out to circular form with a diameter of 6 cm. Each of the disks was sealed into a can 6 cm. in diameter and 7 cm. deep, together with adobe clay after the manner shown in fig. 1. The cans were perforated in the bottom. A layer of filter paper was placed in the bottom of each can, the desired amount of air-dry soil was tamped in, the disks were cemented in place. In three cans the caliche disk was placed at the top, while in the other three more soil was tamped in on top of the disks. The cans were set in a pan

TABLE 1

Evaporation from porous cup atmometer and from disk of caliche 9 cm. in diameter and 3 cm. thick

Readings taken in shade and still air of laboratory. Figures indicate losses per 100 sq. cm. per hour.

	ATMOMETER	CALICHE
Apr. 21, 10.00 p.m.	. .	. . .
Apr. 22, 1.00 p.m.	1 5	.30
Apr. 23, 12.00 noon	1.6	30
Apr. 23, 1.00 p.m.	1 7	24
Apr. 24, 12.00 noon	1 6	31
Apr. 24, 2.00 p.m.	2 0	.25
Apr. 24, 9.00 p.m.	2 0	35
Apr. 24, 11.00 p.m.	2 0	.41
Apr. 25, 12.00 noon	1.7	.31

TABLE 2

Amounts of water lost by caliche lying in or above moist soil, and moisture content of caliche and soil after 12 days

The numbers refer to the experimental arrangement shown in fig. 1. Evaporation in grams, moisture contents in percentages on dry weight.

	1	2	3	4	5	6
Total evaporation in 12 days	17 3	19.0	14 8	40 3	50 2	35 3
Final moisture content of caliche disks	10.0	7 7	5.4	3 2	3 0	4 8
Final moisture content of soil below caliche.	21.5	24.0	23 2	8 3	8.5	12 2
Final moisture content of soil above caliche	. .	. . .	....	4 6	4.0	3 5

of water, 1 cm. deep, for 10 days to allow both soil and caliche to reach their maximum water content. They were then removed, dried, and set into shallow tin lids, 7 cm. in diameter, containing hot paraffine, in order to seal the perforations. The cans were kept indoors and weighed daily from April 25 to May 4 and finally on May 7. At the close of the weighings, the cans were opened, and determinations were made of the moisture content of each piece of caliche and of each of the 10 separate bodies of soil. The resulting data are shown in table 2.

In the cans numbered 1, 2, and 3 the caliche layers were placed on the surface. The amounts of evaporation were less from these cans than from the ones with soil exposed at the surface. The amounts of evaporation from cans 1, 2, and 3 did not differ by amounts proportional to the relative thickness of the three layers, but were of the same general order of magnitude. The percentages of moisture remaining in the disks at the close of the experiment were not proportional to the thickness of the disks, although the 3-cm. disk in can 1 contained the highest amount. Although care was taken to select disks which appeared to have uniform texture it is obvious that differences existed in the minute structure of the different ones. Any number of samples of caliche may be expected to differ in texture, and consequently in their water content and rate of evaporation. The bodies of soil beneath the caliche disks showed very different percentages of residual moisture. In the three cans with caliche on the surface (1, 2, and 3) the percentages of soil moisture remained high (21.5 per cent, 24.0 per cent, 23.2 per cent). In the cans containing disks which were overlaid by soil (4, 5, 6) the three exposed bodies of soil were naturally reduced to low percentages, and the three bodies lying be-

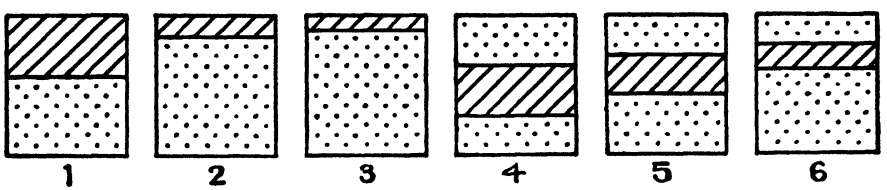

FIG. 1. ARRANGEMENT OF CALICHE IN CANS OF SOIL USED FOR DETERMINING RATE OF EVAPORATION

neath the caliche were also reduced to percentages much lower than those reached by the bodies of soil which were covered by disks which had no soil above them. In terms of figure 1 the moisture content of the lower soil bodies in cans 4, 5, and 6 was lower than in cans 1, 2, and 3. This may be taken to indicate that caliche will convey water more rapidly from the soil beneath it to soil above it than it will from subjacent soil to the atmosphere. The explanation of this may lie in the action of caliche as a semi-permeable membrane.

INFLUENCE OF CALICHE ON PLANTS

The presence of layers of caliche in the soil is of importance to plants from a purely physical standpoint through the mechanical interference with root development and through the modification of the normal movements of water in the soil.

From a chemical standpoint the presence of the softer type of caliche serves to maintain a high concentration in the soil solution. It is not the primary agent in this connection, since caliche is itself due to the prolonged accumulation of salts, and forms in the first instance only in soils so situated topographi-

cally as to become charged with salts leached from the higher levels of the same drainage system.

The interference with roots is easily demonstrated by excavations, in which roots are found running horizontally for as much as 3 to 5 m. along the surface of extended layers of caliche. After penetrating cracks in the first layer the root may be found running horizontally again along the surface of the second layer, not infrequently in the opposite direction to that which it first traversed. In localities on the bajada of the Tucson Mountains with from four to seven well-defined layers of caliche, the roots of *Acacia paucispina* have been found at a depth of 2.5 m., underlaid by poorly stratified and relatively soft caliche.

Several experiments have been made to determine whether roots etch the surface of caliche or penetrate the layers by means of acid excretions. Small pieces of caliche were smoothed on one side, given a high polish, and cemented into flower pots. Corn, beans, cotton, and creosote bush (*Larrea*) were planted in the pots and allowed to remain from 3 to 20 months. No evidences of root etching were found. Larger plants of *Larrea* were raised in pots with unpolished pieces of caliche sealed in place near the bottom, and were allowed to remain for 2 years under greenhouse conditions. There was a large development of roots but no penetration of the caliche layers.

With reference to water movements caliche is a distinct deterrent to the penetration of water from the surface to deeper layers of the soil. When water has finally reached the lower levels, however, the hard layers of the stratified type are very effective in its retention. Field evidence indicates that between favorable and unfavorable functions the balance is in favor of the latter, and that the moisture conditions of the soil would be more favorable to plants without caliche than with it. When the obstruction to root development is taken in conjunction with the influences on soil-water movement, a strong case is developed against caliche as a soil constituent.

Some evidence of the rôle of caliche in relation to plant development may be obtained in the Tucson region, where there are extensive areas both with and without it. On the nearly level outwash plains where caliche is well developed the predominant plant is the creosote bush (*Larrea tridentata* = *Covillea tridentata*, Zygophyllaceae), an evergreen shrub. Perennial plants of secondary abundance in caliche areas are *Acacia paucispina*, *Krameria glandulosa*, *Prosopis velutina*, and several species of cacti. The depth of the soil overlying the caliche can be predicted with a fair degree of accuracy by the density of the stands of *Larrea* and by the height of the bushes, which varies from 50 and 75 cm. in the most shallow soils to 100 and 200 cm. in deep soil. In the Tucson region *Larrea* is infrequent or absent in areas where caliche is not found, although this is not true of it in other parts of its geographical range. When it occurs on soils free of caliche it attains the greater height just mentioned, branches more freely, and bears heavier foliage. On areas free of caliche there is also a heavier stand of *Acacia*, *Parkinsonia*, *Olneya*, *Prosopis*, and other woody perennials. Areas of *Larrea* in Cochise County, Arizona, at an eleva-

tion of 4,000 feet (1,220 m.), with an approximate rainfall of 14 inches (355 mm.), but with soil heavily impregnated with caliche 15 to 25 cm. from the surface, have been compared with areas in the District of Altar in Sonora, Mexico, at an elevation of 1,600 feet (488 m.), with an annual rainfall of 9 inches (228 mm.), but on soil free of caliche. In spite of the higher rainfall in Arizona, the stand of *Larrea* is much heavier and the plants much taller in Sonora. It is apparent from these comparisons that the low and open character of the vegetation, associated with desert conditions and commonly attributed to inadequate water supply, is due in many parts of Arizona and adjacent states to the influence of caliche in restricting root development, reducing the penetration of water, and assisting in the maintenance of a high salt content in the soil solution.

The soft type of caliche has no such effects on the water relations of the soil and the development of the root system as those that have been ascribed to the hard type. Many localities have been examined in which the amount of soft material was greatly in excess of the hard layers. Water movements and root distribution in the thick beds of soft material are more nearly what would be found in a normal soil. The fact that the hard and soft types are almost invariably associated, even when the latter is by far the more abundant, renders it difficult to obtain field evidence on the influences which the soft type may exert on the growth of plants. In order to obtain experimental evidence in soils highly impregnated with soft caliche without having to deal with the known effects of the hard layers, a series of cultures of *Larrea* was arranged and allowed to run for 20 months. Three widely different soils were selected: adobe clay, a granitic loam, and sand. Each of these was used pure and also with varying amounts of finely divided caliche. The amounts of caliche were 10, 25, 50, 75, and 90 per cent. The six soils were made up in duplicate for each of the three types, making 36 cultures, to which were added two pure caliche units. The soils were placed in large carbide cans 12 inches (30 cm.) in diameter and 22 inches (56 cm.) deep. Three plants of Larrea between 5 and 10 cm. high were set in each can in August, 1927. Some replanting was done in September, 1927, to replace plants which died as result of removal. The cans were placed in a lath shelter and were watered lightly once a week except in the winter and summer rainy periods. Out of the 114 plants there were 96 survivals at the termination of the experiment in April, 1929; of the 18 deaths 11 were in cultures with 90 per cent caliche and 10 per cent soil, and one was in the pure caliche.

In table 3 are shown the heights and dry weights of the plants reared under the 19 cultural conditions. Because of the uniform size of the plants at the beginning of the series and the length of time given them for growth, no allowance has been made for the small differences existing at the start. The height figures are averages of the six plants raised under the same conditions, with only two to five plants surviving in six cases. The dry weights are the average for single cultural conditions, with the same limitations due to incompleteness of the series at the close.

With respect to growth in height it will be seen in table 3 that the poorest growth, 20 cm., was made by the plants in pure caliche. In each of the cultures in which 10 per cent of sand, loam or clay was mixed with caliche the growth was from two to two and a half times as great. In the sand series the growth in height in pure sand was not so great as in the mixture 10 per cent sand + 90 per cent caliche. The greatest growth in this series was found in the cultures approximating an equal mixture of sand and caliche, which is to be

TABLE 3

Average height and dry weight of plants of Larrea *raised in sand, loam, and clay with different percentages of soft caliche, together with the percentages of calcium oxide and magnesium oxide in the ash and their ratios*

	HEIGHT	DRY WEIGHT	CaO	MgO	CaO/MgO
	cm.	*gm.*	*per cent*	*per cent*	
Sand:					
Pure sand	43	14 15	33 62	2 90	11.59
Sand + 10 per cent caliche	56	13 89	39 19	3 48	11.25
Sand + 25 per cent caliche	64	13 70	27.36	2 28	12 00
Sand + 50 per cent caliche	64	16 33	35 05	2 98	11.77
Sand + 75 per cent caliche	49	6 14	32 04	4 38	7 31
Sand + 90 per cent caliche	50	4 49	32 32	5 08	6 36
Loam:					
Pure loam	63	14 37	19 50	3 32	5 87
Loam + 10 per cent caliche	73	13 45	30 25	4 34	6 97
Loam + 25 per cent caliche	57	11 42	31 25	3 90	8 06
Loam + 50 per cent caliche	60	11.98	31 30	5 10	6.14
Loam + 75 per cent caliche	48	5 42	31 70	4 90	6 46
Loam + 90 per cent caliche	54	6 26	25 53	5 00	5 11
Clay:					
Pure clay	69	29 21	31 56	3 86	8 17
Clay + 10 per cent caliche	86	21 76	20 24	2 06	9.82
Clay + 25 per cent caliche	75	18 69	31 30	3 97	7 88
Clay + 50 per cent caliche	67	18 21	28 97	3.91	7 46
Clay + 75 per cent caliche	49	12 76	27 73	3 87	7 16
Clay + 90 per cent caliche	40	7 02	34 30	6 02	5 69
Pure caliche	20	8 00	33 65	5 74	5 86

attributed to an increase in the water-holding capacity of the sand due to the caliche.

In the loam series the greatest growth was found in 90 per cent loam + 10 per cent caliche, while there is evidence of a tendency for growth to be less with increasing percentages of caliche.

In the clay series the greatest growth was again registered in the culture 90 per cent clay + 10 per cent caliche, and a marked decrease was found in the amount of height growth with increasing percentages of caliche.

The figures given in table 3 for dry weight of tops in the several cultures are

more significant of the growth of the plants than are the data on height. The average dry weight per plant was greater in the pure caliche cultures than in any of the cultures with soil plus 90 per cent caliche. The differences are small, however, and of little significance in comparison with the differences exhibited between all dry weights for cultures with high percentages of caliche and those with low percentages. In the sand series the greatest dry weight, as well as the greatest height growth, occurred in the culture 50 per cent sand + 50 per cent caliche. With a further increase in the percentage of caliche there was a sharp falling off in the dry weight. In the loam series the greatest dry weight was found in the plants in pure loam, with a slight decrease accompanying the increased percentages of caliche. In the clay series the dry weight fell rapidly and consistently with increasing caliche. The maximum dry weight per plant was 16.33 gm. in the sand series, 14.37 gm. in the loam series, and 29.21 gm. in the clay series. Considering each series separately and comparing the dry weight in the cultures with soil plus 90 per cent caliche and those in the pure soils, one sees that the high content of caliche reduced the growth to less than one-third in sand, less than one-half in loam, and less than one-fourth in clay.

A complete analysis of the soil conditions responsible for the differences in growth is not possible in connection with this preliminary investigation. No determinations of soluble salts were made, but this condition is being followed in detail in work now in progress. The determinations of pH for the loam series gave readings ranging from 8.05 for the pure loam to 8.60 for the culture with 10 per cent loam + 90 per cent caliche. In the clay series the readings were 8.0 throughout the series. The moisture equivalents of the basic soils are 4.29 for the sand, 9.00 for the loam, 25.21 for the clay, and 25.56 for caliche material under $\frac{1}{30}$ inch diameter. These figures mean that under the uniform but low water supply given to all the plants the comparative growth and dry weight in the three soils are to be attributed to the differences in available water. The differences between the growth in clay on the one hand and loam and sand on the other are greatest in the pure soils and those with low percentages of caliche, and become less as the amounts of caliche are increased and the texture of the soil in the three series is thus rendered more uniform.

The calcium and magnesium contents of the ash of the stems and leaves were determined to show the influence of the different soil mixtures on the absorption of these elements. Samples were prepared from each of the cultures, as raised in duplicate, but only one determination was made for each cultural condition, samples containing equal parts by weight of the pulverized material being used.

The material was prepared for analysis by the methods of the Association of Official Agricultural Chemists (1924 revision, p. 30). For determining the calcium the tentative volumetric method given in section 6, page 41 of that publication was followed and the tentative gravimetric method for magnesium given in section 7, page 42 was adopted up to the point where the sample is ignited before weighing. For the final determination of magnesium the

volumetric method of Handy (1908) as given in Scott (1922, ed., 2, revised) was used. The results of these analyses are presented in table 3 as percentages of the weight of ash used for the determination. The Ca/Mg ratios are also given.

In table 4 are shown the percentages of CaO and MgO and the Ca/Mg ratios found for aerial portions of *Larrea* bushes growing on four characteristic areas of this plant. The same methods were used in obtaining these data, which were obtained in connection with another piece of work not yet published. On two of the areas caliche was very prevalent, whereas on areas III and IV caliche was absent to a depth of 4 feet or more.

Inspection of these data shows that the presence of caliche in the soil does not reduce and may possibly increase the absorption of both calcium and magnesium. The soil type, however, seems to exert a more pronounced influence upon the percentages of calcium and magnesium found in the ash, the sand series yielding the highest values, in general, followed by the loam and clay series respectively. From the results obtained for the pure caliche cultures it

TABLE 4

Percentages of calcium oxide and magnesium oxide, and their ratios, in the ash of Larrea *from two localities with caliche near the surface and two without it*

	AREA	CaO	MgO	CaO/MgO
Caliche near surface	I	22 45	6.12	3.67
	II	16 86	4 77	3 66
Caliche absent	III	15 43	4 80	3.22
	IV	24 90	6.28	3.96

is evident that sufficient calcium and magnesium were available to satisfy completely the needs of the plants. In only three of the "mixed" cultures was the calcium content greater than in the pure caliche culture, and in only one instance was the magnesium content greater.

It is evident that the amounts of calcium and magnesium available to plants in normal desert soils are great enough to give only slight increases, if any, in the amount of these elements in the ash in the case of plants reared in soils with high percentages of poorly available calcium. The conclusion is reached that the physical properties of the soils highly impregnated with calcareous material are more important than the chemical ones in causing the reduction in growth found in the case of *Larrea*.

It is interesting to note that the calcium-magnesium ratios are consistently greater for the plants grown in culture than for those which developed under natural conditions. Since the amounts of magnesium absorbed are nearly equal in the two cases the difference is due chiefly to the greater amounts of calcium taken in by the cultured plants. The latter were much younger and

smaller plants and their roots systems were neither so extensive nor probably so well established. It may be that young *Larrea* shrubs have a higher calcium requirement than older plants. Calcium is thought to be closely related to the formation of hydrophilic colloids within a plant and the hydrophilic colloids are, in turn, intimately concerned in drought resistance. The formation of relatively larger quantities of hydrophilic colloids in young plants would serve as a safeguard against drought until they became thoroughly established.

The calcium content of *Larrea* is relatively high and shows a general agreement with the percentages found by Richardson (6) for certain dune plants from the south shore of Lake Michigan. *Artemesia caudata* contained 35.47 per cent calcium oxide; *Prunus pumila* 44.13 per cent; and *Quercus coccinea tinctoria* 28.86 per cent according to his analyses.

The number of ash analyses which could be included in this investigation was too small to establish definite conclusions in regard to the influence of the various soil conditions studied upon calcium and magnesium absorption. However, the data obtained indicate that the presence of soft or broken caliche probably either increases the calcium content or at best does not lower it; that the physical properties of the soil are more important for the growth of *Larrea* in the desert soils used, than is the chemical constitution, and that the calcium content is relatively high and may have a direct bearing upon the drought resistence of the creosote bush.

SUMMARY

In the southwestern United States, layers of calcareous hardpan, or "caliche," are abundant over extensive areas.

The formation of caliche is due primarily to the interrupted penetration of rain water under arid conditions. Various modifying factors are involved in the formation of caliche in different situations. It occurs in hard layers and in amorphous masses, in both of which forms it has an important influence on the physical and chemical properties of the soil and on the development of the root system.

The maximum water content is very low for the hard layers of caliche (3.2 to 6.5 per cent) but higher for the softer masses (12.9 to 17.3 per cent). Even the thinnest layers of caliche greatly retard the upward or downward movement of water in the soil. Evaporation from caliche which is underlaid by water is less than from an equal surface of a porous cup atmometer.

Considerable differences of moisture content may exist in bodies of soil separated by a layer of caliche. Caliche will convey water more rapidly from soil beneath it to soil above it than it will from soil to the atmosphere.

Roots are unable to penetrate the silicified hard layers of caliche and their distribution is thereby seriously interfered with. The abundance of caliche in the soil of bajadas appears to be an important condition in determining the low and open character of the vegetation, chiefly restricted in such localities to the creosote bush, *Larrea tridentata* (= *Covillea tridentata*).

Cultures of *Larrea* were made in 18 types of soil, varying in texture and percentage of soft caliche. The poorest growth was made in pure caliche; the best growth in soils containing equal amounts of caliche and sand, loam, or clay respectively, or these soils with lower percentages of caliche. The dry weight of the plants decreased with increasing percentages of caliche.

The percentages of CaO in the ash of the cultured plants varied from 20.24 to 39.19 and those of MgO from 2.06 to 6.02. The varying amounts of caliche in the soils were without influence on the amounts of CaO and MgO in the ash. This result has been confirmed by data from field material taken on soils with and without surface caliche.

The fact that the Ca/Mg ratio of the young cultured plants is consistently higher than that of mature plants growing in their natural habitats is due to the higher CaO, and may indicate greater drought resistance on the part of the young plants.

The chemical properties of highly calcareous soils appear to be of less importance than the physical in relation to the growth of *Larrea*.

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PLATE 1

FIG. 1. Caliche from uppermost layer (left). The hard surface 1 cm. thick is underlaid by softer material. Hard layer of caliche 5 cm. thick (right).

FIG. 2. Stone covered with caliche in stalactite form, from roof of small cavity in the soil (left). Recemented type of caliche with many air spaces (right).

FIG. 1

FIG. 2

INFLUENCE OF RYE AND OAT STRAWS UPON THE GROWTH AND YIELD OF CERTAIN VEGETABLES[1]

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Among the soil problems which arise in connection with truck farming the lack of sufficient quantities of organic matter is of great importance. Much work has been done at the Rhode Island Agricultural Experiment Station in connection with the efficiency of substitutes and supplements for stable manure in vegetable rotations. Fertilizer chemicals combined with stable manure have given better yields than stable manure alone. Green manure and fertilizer chemicals have failed to produce as high yields as fertilizer chemicals and stable manure. Combinations of stable manure, green manure, and fertilizer chemicals have proved very efficient (1, 2).

In addition, it was thought that another method of substitution might be the use of straws coupled with ample quantities of fertilizer chemicals. To this end experiments were carried on over a period of years by growing certain vegetables in pots using soils to which varying amounts of straw were applied.

EXPERIMENTAL METHODS

The crops were grown in Wagner pots from seed or small transplants to marketable maturity. Table 1 gives data showing variety, soil source, planting and harvest dates, and number of plants in each pot. There were three pots for each culture. Each pot was filled with carefully screened soil in which the weighed quantities of organic matter were mixed. Two pounds of beach sand was also mixed into the soil for each pot. The soil (Merrimac silt loam) came from two sources. Plat 102 is an agronomy plat which has had chemical fertilizers annually since 1904 for the growing of vegetables. The pH of this soil was 5.2 and the organic matter content accumulated from green manure and crop residues was calculated to be 1.64 per cent. The soil which was used with oat straw was taken from an uncultivated area beside the market garden plats and had a pH of 5.5 and an organic matter content of 4.57 per cent.

The organic matter used was from four sources. Rye straw I, which was, 2 years old and partially decayed, was taken from a station straw stack. Rye straw II came from the base of an old rye stack on a nearby farm and showed little indication of soft decay. Rye straw III was from the same lot as

[1] Contribution No. 427 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

rye straw II but had been allowed to decay a year longer. The oat straw came from a nearby farm and was taken from the base of an old stack which had

TABLE 1

Miscellaneous data connected with growth of crops in Wiley pots

CROP	PLANTED FROM	NUMBER OF PLANTS	SOIL SOURCE	DATES		VARIETY
				Planting	Harvest	
Rye Straw I:						
Lettuce....	Plants	8	Plat 102	June 20	Aug. 9	Cos
Celery.....	Plants	8	Plat 102	June 22	Oct. 22	Golden Plume
Beets.....	Seed	7	Plat 102	June 20	Oct. 29	Early Wonder
Carrots....	Seed	6	Plat 102	May 15	Oct. 31	Danvers Half Long
Onions.....	Plants	12	Plat 102	June 19	Oct. 30	Yellow Globe Danvers
Rye Straw II:						
Lettuce....	Plants	7	Plat 102	May 23	July 16	
Celery.....	Plants	7	Plat 102	June 5	Sept. 19	
Beets.....	Seed	8	Plat 102	May 21	Sept. 24	
Carrots ...	Seed	9	Plat 102	May 22	Oct. 2	
Onions.....	Seed	10	Plat 102	May 21	Oct. 3	
Spinach...	Seed	10	Plat 102	May 22	June 30	Savoy Bloomsdale
Rye Straw III:						
Lettuce...	Plants	7	Plat 102	May 29	July 15	
Celery....	Plants	7	Plat 102	June 19	Oct. 2	
Beets.....	Seed	8	Plat 102	May 29	Sept. 18	
Carrots ...	Plants	10	Plat 102	May 29	Oct. 26	
Onions.....	Plants	10	Plat 102	June 1	Oct. 2	
Spinach....	Seed	10	Plat 102	May 29	July 9	
Oat Straw:						
Lettuce....	Seed	8	Market Garden Roadway	May 17	July 9	
Celery.....	Plants	7	Market Garden Roadway	June 11	Sept. 10	
Beets.....	Seed	7	Market Garden Roadway	May 17	Sept. 20	
Carrots....	Seed	8	Market Garden Roadway	May 17	Oct. 2	
Onions.....	Seed	10	Market Garden Roadway	May 17	Oct. 24	
Spinach....	Seed	10	Market Garden Roadway	May 17	July 1	

been exposed to the weather for some time. This material was black and soft and appeared to be well decayed. Table 2 gives the analyses of these organic

matter materials. Varying amounts of these materials were applied to groups of three pots. These amounts were as follows, in tons per acre:

	LOW	MEDIUM	HIGH
Rye I	4 4	8 8	13.3
Rye II	3 3	13 3	20
Rye III	3 3	13 3	20
Oat	3 3	13.3	20

The fertilizer applications were made in two quantities in all cases with the exception of rye straw I. In that year an attempt was made to determine the optimum fertilization, and three levels were used. Table 3 shows the amounts of the initial fertilization and the totals of such additional applications as were made during the growing periods. At various times during the growing season, soil nitrate determinations were made and in accordance with these results

TABLE 2

Composition of organic materials

ORGANIC MATERIALS	WATER	ORGANIC MATTER	NITROGEN*	P_2O_5*	K_2O*
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Rye Straw I	61 8	20 8	1 37	0 62	0.53
Rye Straw II	65 4	31.7	0 72	0 26	0.54
Rye Straw III	72 2	23 1	1 02	0 28	0 43
Oat Straw	77 3	19 1	2 58	0 90	0 68

* Dry matter basis.

additional applications of nitrate of soda were made when needed. Lime was also supplied at the rate of 2 tons of limestone per acre.

Because it was realized that the moisture content of the soil was likely to be affected by the quantity of organic matter supplied, with rye straw I an attempt was made to evaluate this factor. Table 4 gives the yields with 15, 22, and 30 per cent soil moisture. In so far as was possible these levels were maintained by watering and weighing the pots at the same time each day. The moisture levels were adjusted by adding enough water to bring the pot up to a predetermined weight.

Determinations of moisture on the agronomy field plats have shown that the average range in 1929 and 1930 was between 17.7 and 27.0 per cent (6). In consideration of this fact and of the data given in table 4, it was decided that for the other three organic sources the soil moisture should be made up to 25 per cent, thus somewhat approximating field conditions.

All crops were grown in the summer glass house in pots on small trucks which were wheeled out of doors on dry days. After harvest the roots were sepa-

rated from the tops and weighed. Then both portions were dried and the air-dry weight taken. Selected samples were ground and stored for analysis.

DISCUSSION OF EXPERIMENTAL RESULTS

In similar experiments a usual procedure is to attempt to control all of the known factors with the exception of one which is left free to operate uncontrolled. If it is not possible to achieve control of a factor an attempt to meas-

TABLE 3

Applications of fertilizer in grams per pot

Fertilizer applied at planting time

	REGULAR				HIGH			
	Rye I*	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat
Leuna saltpetre	0 7	0 7	0.7	0 7	1 0	1 0	1 0	1.0
Hoof meal	4 0	4 0	4 0	4 0	13 7	4 0	4.0	4 0
Superphosphate	3.5	3 5	3 5	3 5	5 25	5 0	5 0	5.0
Thomas slag	3 5	3 5	3 5	3 5	3 5	3 5	3 5	3.5
Sulfate of potash	2.2	2 5	2 5	2.5	2 8	3 0	3 0	3 0

Additional fertilization during growth of crops

	RYE I			RYE II		RYE III			OAT	
						$NaNO_3$				
	$NaNO_3$	K_2SO_4	Superphos	$NaNO_3$	K_2SO_4	0 and low O.M.†	Medium and high O.M.	K_2SO_4	$NaNO_3$	K_2SO_4
* Lettuce	0 5	0.5	2 0	3.0	...	2.0	4 0	..	0.5	0.5
Celery	1 5	0.5	2 0	6 0	2 0	8.0	10 0	1 0	3.0	1.0
Beets	2 0	0 5	2 0	7 0	2 0	8 0	11 0	1 0	4 5	2.0
Carrots	1 5	0 5	2 0	4 0	1 0	6 0	8 0	1 0	3 5	1.5
Onions	1 5	0 5	2 0	3 0	....	3 0	6 0	1.0	2 5	0.5
Spinach	..	...	..	1.0	..	3 0	3 0	....	0 5	0.5

* With rye straw I a medium fertilizer level with the medium organic matter level was used in addition to the regular and high amounts. This consisted of 1.0 gm. Leuna saltpetre, 9.0 gm. hoof meal, 4.55 gm. superphosphate, 3.5 gm. Thomas slag, and 2.8 gm. sulfate of potash.

† Organic matter.

ure the results of its change is often made. In this case the moisture, fertilizer, and organic matter factors were controlled. The results in table 4 indicated that making up the moisture daily to 25 per cent would give an average moisture of approximately 22 per cent and that optimum crops might be expected with this moisture.

For the fertilizer a medium or regular application was chosen (table 3), but in order to determine to what extent the yields were optimum with this fer-

tilizer level a higher quantity was used in all cases with all organic sources except rye I. With such control of these two factors and with environmental factors the same for all the crops in the experiment it was thought that the effects of applications of different quantities of organic matter might be expected to show up in the growth of the vegetable crops.

In table 5 are given the dry weight yields of the various crops as grown with different quantities of the four organic sources. In general a definite increase in yield was noted with the increasing quantities of rye I and oat straw. This did not hold true with rye II and rye III. In the latter years some other factor seemed to be interfering with any effect which the higher organic matter might

TABLE 4

Dry weight yields, in grams, of crops grown with varying moisture and organic levels

Rye I

ORGANIC MATTER APPLIED	LETTUCE			CELERY			BEETS			ONIONS			CARROTS			SPINACH*	
	Per cent moisture																
	15	22	30	15	22	30	15	22	30	15	22	30	15	22	30	25	30
Regular fertilizer																	
No organic matter	.	47	..		39		..	103	..	.	51		..	170	...	51	56
Low organic matter	38	.	70	38	...	115	99		142	50	.	93	90	.	201	58	54
Medium organic matter.	40	51	63	52	129	123	100	121	96	45	57	106	105	193	247	69	69
High organic matter	48	67	60	53	124	135	94	107	114	47	73	151	87	181	201	....	.
High fertilizer																	
No organic matter		...	...		...	...	..	..	...	.	..	...	...	.	...	55	. . .
Low organic matter	33	...	43	44	.	170	99	...	187	51	...	157	111	.	303	61	. .
Medium† organic matter.	49	52	41	49	146	176	95	189	191	48	103	170	89	257	288	73	72
High organic matter.	51	69	64	61	129	158	104	131	129	67	105	148	105	230	254	...	..

* The organic matter source with spinach was oat straw.

† Medium fertilizer (see table 3).

have had. In order to investigate the cause of this effect, nitrate determinations were made on the soil in the pots from time to time. These results are given in table 6. In all cases the nitrate nitrogen content was much lower where the high quantity of organic matter had been added.

The depressing effect upon soil nitrates of undecomposed straw has been noted by Murray (4), Scott (5), and others. Waksman (7) and his coworkers have shown that nitrate accumulation is related directly to the carbon and nitrogen content of the organic matter applied to the soil.

If the nitrogen content is more than 2 or 2.5 per cent, no nitrogen starvation can set in, but ammonia will be liberated. If the nitrogen content of the organic matter is about 2 or 2.5 per cent, a temporary nitrogen starvation may set in lasting a few days followed by liberation of

TABLE 5

Dry weight yields in grams, of crops grown with varying levels of oat and rye straw

ORGANIC MATTER APPLIED	LETTUCE				CELERY				BEETS				CARROTS				ONIONS				SPINACH			
	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat	Rye I	Rye II	Rye III	Oat
Regular fertilizer																								
No organic matter.....	47	120	81	133	39	236	245	250	103	309	324	352	170	397	390	399	51	275	221	250	....	52	56	51
Low organic matter..	. ..	116	86	138	...	216	225	254	...	271	318	360	.. .	384	365	420	....	275	255	315	....	50	54	58
Medium organic matter. .	51	75	87	160	129	181	224	269	121	234	348	380	193	280	408	488	57	219	208	284	....	23	47	69
High organic matter.....	67	54	80	171	124	155	231	302	107	233	332	427	181	236	443	520	73	154	201	365	....	14	44	73
High fertilizer																								
No organic matter.....	....	122	85	135	....	246	243	253	....	351	332	371	....	424	382	414	....	223	219	261	....	53	60	55
Low organic matter.....	....	117	91	135	....	230	246	253	....	323	309	371	....	412	348	436	....	242	214	281	....	47	59	61

ammonia. If the nitrogen content of the organic matter is about 1 per cent or less, a lasting nitrogen starvation may set in which may be corrected by the addition of inorganic nitrogen fertilizers.

Table 2 shows that the nitrogen content of rye II and rye III closely approached 1 per cent or less and this fact connected with the soil nitrate results (table 6) may explain the lower yields with high organic matter in the cases of rye II and rye III. In other words, the decomposing straw kept the soil nitrates so low that the growth of the plant was affected and constituted the

TABLE 6

Nitrate nitrogen, in parts per million, in soil of organic matter pots

DATE OF SAMPLING	BEETS (RYE II)		BEETS (RYE III)		CARROTS (RYE III)		SPINACH (RYE III)	
	No O.M.*	High O.M.	No O.M.	High O.M.	No O.M.	High O.M.	No O.M.	High O.M.
August 1	188	2						
September 4	12	2						
June 15			62	28				
June 23							54	22
July 9			12	8	43	3		

* Organic matter.

TABLE 7

Total nitrogen content, in milligrams per 100 gm. of dry tissue, of crops grown without organic matter and with high organic matter applications

CROP	OATS		RYE II		RYE III	
	No O M *	High O.M.	No O M.	High O.M.	No O M	High O M.
Beets	4,184	6,063	2,119	1,695	5,220	5,549
Carrots	3,425	4,879	1,740	1,019	4,072	4,612
Celery	3,174	4,749	3,184	2,134	4,086	4,223
Lettuce	2,348	3,234	2,840	1,146	2,166	2,198
Onions	3,079	5,338	3,558	1,967	3,608	3,904
Spinach			1,649	328	2,257	1,521

* Organic matter.

unknown limiting factor which masked any effect on yield that increasing quantities of organic matter might have produced. Further evidence that the supply of available nitrogen may have been the limiting factor is given in table 7.[2] It will be noted that with the highest quantity of oat straw the total crop nitrogen was consistently greater with all the crops, whereas in the case of rye II the opposite results are found. With rye III the large applications of nitrogen of that year balanced the detrimental effects of the organic matter with

[2] The chemical analyses were made by Mr. J. B. Smith and his co-workers.

lettuce, beets, and carrots (table 5). The plants which received the high application of organic matter had some reserve nitrogen, as shown by table 6. The results with the other crops—onions, celery, and spinach—are not so conclusive.

With rye I and oat straw the total nitrogen content of the straws was perhaps sufficient to supply the requisite nitrogen for decay without interfering with the growth of the plants.

Some writers have doubted that extreme cases of nitrogen starvation brought about by resistant organic matter in the soil, could be corrected by applications of nitrogen fertilizers. Hill (3), using pure cellulose, got little effect with an 8-ton application of sodium nitrate with corn. In this experiment the nitrate nitrogen deficiency with rye II and III having been with additional applications of sodium nitrate were made (table 3). This was tried particularly with rye III. It will be noted, however, that even with 3,030 pounds of nitrate of soda per acre with beets the yield from high organic matter was barely held up to the no-organic matter yield. Thus in this experiment, although benefit was derived from the use of additional nitrogen, it is doubtful whether the unruly nitrogen starvation factor could have been entirely eliminated by additional fertilizer applications.

SUMMARY

Further evidence is given to show the inhibitory effect upon plant growth of large quantities of poorly decayed organic matter. By the use of various levels of rye and oat straws of different composition and stages of decomposition an attempt was made to determine the value of these sources of organic matter in connection with the growth of lettuce, celery, beets, carrots, onions, and spinach. These pot tests seem to show the following:

Such resistant organic matter sources have doubtful value as substitutes for stable manure. This is especially true where the nitrogen content of the straws is very low.

Evidence is given to show that, although it may be possible to balance the inhibitory effect which accompanies the decomposition of low nitrogen straws by very large applications of nitrogen, these are of necessity so large as to be out of reason for field practice.

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A STATISTICAL STUDY OF NITROGEN FIXATION BY CLOVER PLANTS[1]

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In 1869, Galton applied to the problem of biological inheritance the mathematical laws of probability, the practical application of which had hitherto been of use only in life insurance and gaming; thus were laid the foundations of the science of biometry. Since that time under the leadership of Galton, Pearson, Yule, Fisher, and others this branch of statistics has been developed into a powerful tool which is becoming more and more indispensable to the research worker in the biological sciences. At present the progress of biometry suffers from two handicaps, viz., lack of use and misuse. All too few biologists avail themselves of the many valuable methods at their disposal for analysis of data obtained in the laboratory or field; until this is done, the statistical methods developed will suffer from an academic formality and idealization which restricts their application to actual data. The success of the Rothamsted Experimental Station in overcoming this handicap lends point to the argument. On the other hand, the misapplication of statistical formulas to observations is primarily due to the carelessness of the user in failing to ascertain, or to understand, the principal assumptions involved in the derivation of the formulas. This unfortunate circumstance will be largely eliminated when the research student acquires a proper acquaintance with statistical methods and thus disarms those critics who carp at the application of anything resembling mathematics to the so-called "descriptive sciences."

As an example of the value of statistical analyses of data this study of the nitrogen fixation process in red clover is presented. In the experiments to be described, data were gathered from 200-odd plants. Because of the large number and the variability in the measurements, little information could be gained by a study of the data without a statistical analysis. However, when subjected to such an analysis, quite definite conclusions were apparent. The primary purpose in the experiments was to obtain some information in regard to the distribution of several variables characteristic of the fixation process. This knowledge was desired in order to apply the proper statistical treatment to data obtained in future experiments made under conditions similar to the ones described. Naturally the specific conclusions reached are more or less restricted to experiments thus circumscribed, but the general nature of the results may be of interest to many workers engaged with the problem of nitrogen fixation in the leguminous species. Similar studies under different conditions, e.g., in the field, or with different species, are desirable not only for their statistical value but because they should elucidate some of the interrelationships that obtain in the fixation process. In addition to the practical benefits, the methods of attack and the nature of the questions answered by an analysis of this kind might interest other research workers irrespective of their concern with the specific problem studied.

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EXPERIMENTAL

Information was desired on the statistical characteristics of two populations, viz., clover plants grown in the open in the greenhouse and plants grown in more easily controlled culture solutions. For this reason the experiment was divided into two parts according to substrate on which the plants were grown: (*a*) nitrogen-poor sand plus modified Crone's solution, (*b*) nitrogen-free Crone's agar. In both parts aqueous suspensions of *Rh. trifolii*, strain 209, prepared from a 48-hour agar slant, were used for inoculation. The technique used in setting up the experiments was that described by Hopkins, et al. (5).

Growth of plants in agar

Plants were grown in 800-cc. glass bottles, each containing 250 cc. of Crone's agar medium adjusted to pH 7.0 by the addition of about 10 gm. calcium carbonate. Six germinating clover seeds were placed in each bottle in a circle, care being taken that the distance between them was fairly uniform. In this way 60 bottles were prepared, of which 40 were inoculated with *Rh. trifolii;* the other 20 were kept uninoculated as controls. Litmus milk was used to test the sterility of the seed and the purity of the inoculum.

At the time of harvest, nodules and leaves were counted, the length of the roots and tops was measured, and then the plants were placed in 20-cm. test tubes with 2 cc. conc. H_2SO_4 and kept until they could be analyzed for total nitrogen.

Growth of plants in sand

Plants were grown in 1-gallon earthenware jars each containing 2,500 gm. of nitrogen-poor sand with which 5 gm. of sterilized $CaCO_3$ was well mixed. Each jar was provided with a glass tube about 2.5 cm. in diameter, leading to within 3 cm. of the bottom and extending about 10 cm. above the top of the jar. This tube was closed at the top with cotton and through it sterile water or modified Crone's solution was added to the sand.

After sterilization at 15 pounds pressure for 8 hours, each jar was seeded with 10 clover seeds under aseptic conditions. Thirty such jars were inoculated with *Rh. trifolii*, and fifteen were left uninoculated as controls. The surface of the sand was then covered with a 5-mm. layer of sterile ground cork to reduce contamination from the air. Through the tube 200 cc. of modified Crone's solution and 200 cc. of sterile distilled water were added. All jars were watered daily with the same quantity of water, the quantity being varied as the weather required.

Nitrogen determination

Total nitrogen was determined by a micro-method similar to those described by Chiles (1) and Walters (9). Each plant was digested with the usual Kjeldahl reagents, and the digest nesslerized directly. As suggested by Looney (6), gum

ghatti solution prepared according to the directions of Folin (3) was used as a protective colloid in the nesslerization.

This micro-nitrogen determination was checked against the regular macro-determination generally used in this laboratory, as follows: Sets of 200 and 300 clover seeds, accurately counted, were digested and analyzed by the macro-Kjeldahl method. The results obtained were used to calculate the average nitrogen content per seed. By the micro-method already described, seeds were analyzed in lots of two, three, or five, and the results compared with those obtained by the macro-methods. The average nitrogen content by the micro-method was 0.0975 mgm. per seed; that by the macro-method, 0.0985 mgm.

The reliability of the method was further examined by a series of recovery tests. For each series five seeds were digested, the digestion mixture was diluted to 100 cc., 20 cc. of which was nesslerized directly. To a second aliquot of 20 cc., 0.05 mgm. of N in the form of $(NH_4)_2SO_4$ was added; this mixture was then boiled, digested, and, after cooling, nesslerized. To a third aliquot of 20 cc. of the same digested sample, 0.10 mgm. $(NH_4)_2SO_4$-N was added, and the total nitrogen was determined after boiling and digestion. The latter is, in fact, not a true recovery test, because the quantity of nitrogen to be recovered is already in the form for direct nesslerization without previous digestion. The procedure was believed, however, to be a fair check on the micro-method. The recovery of added nitrogen ranged from 94.0 to 104.0 per cent. Though the error appears fairly large, it is, in consideration of the small quantities to be recovered, small. Variations in nitrogen content of the plant were so great, that an error of +4 or −6 per cent would be of little consequence to the final results. Because of the close checks with the macro-Kjeldahl method and because of the fair recovery tests, this micro-nitrogen method was adopted for the work.

DISCUSSION OF RESULTS

The plants grown in agar were placed in the greenhouse February 24, 1931, and harvested 94 days later. At the time of harvest, the plants were tall and inclined to be spindly and pale green; the leaves were numerous but small. The plants grown in sand were seeded March 4, 1931, and harvested after 71 days. These plants were tall, sturdy, dark green, and had large leaves. Summaries of the observations and measurements are given in tables 1 (agar series) and 2 (sand series). The nitrogen content of the uninoculated controls in the agar series varied from 0.16 to 0.30 mgm. This entire range was just equal to that of each *class* of the inoculated plants; therefore, the mean value of nitrogen in the controls was used to determine the *nitrogen fixed* in this series. The same was true of the sand series except that the controls ranged from 0.30 to 1.00 mgm.; this difference in the nitrogen content of the controls lies in the small quantity of nitrate nitrogen available to the plants grown in sand. The number of nodules, length of stem, and length of roots were divided into classes whose limits were so chosen that no actual measurement could fall on a class

limit; e.g., the class limits of the length of stem and roots were of the type 5.25 to 6.25 cm., but the measurements were made only to the nearest 0.5 cm. The data were grouped in all cases so that 10 to 12 classes would be formed of the 200-odd observations; this required that the class range should be somewhat more than one-quarter of the standard deviation as advised by Fisher (2, p. 50). However, in order to obtain a sufficient number of classes (12, p. 79), the grouping adopted was necessary; the error in the estimates of the parameters calculated from the data so grouped is about 2 to 3 per cent at the

TABLE 1

Inoculated clover plants grown in agar

NITROGEN FIXED		NODULES		LENGTH OF STEM		LENGTH OF ROOT		LENGTH OF ROOT / LENGTH OF STEM		LEAVES	
Range	Frequency	Range	Frequency	Range	Frequency	Range	Frequency	Range	Frequency	Number	Frequency
mgm.	*no.*	*no.*	*no.*	*cm.*	*no*	*cm.*	*no.*	*no.*	*no.*	*no.*	*no.*
<0.30	3	<7 5	21	<4.25	5	<9.25	11	0 0 -1 01	14	4	3
0.30–0.45	5	7.5–12.5	49	4.25– 5 25	3	9.25–11 25	18	1.01–1 21	22	5	4
0 45–0.60	9	12.5–17.5	45	5.25– 6.25	2	11.25–13.25	22	1.21–1.41	15	6	5
0.60–0.75	22	17.5–22.5	37	6.25– 7.25	10	13.25–15.25	25	1.41–1.61	30	7	20
0.75–0.90	29	22.5–27.5	15	7 25– 8 25	20	15.25–17.25	28	1.61–1.81	25	8	22
0.90–1.05	25	27.5–32.5	12	8.25– 9.25	21	17 25–19 25	30	1.81–2.01	20	9	38
1.05–1.20	23	32.5–37.5	5	9.25–10.25	47	19.25–21 25	13	2.01–2 21	22	10	58
1.20–1.35	20	37.5–42.5	6	10 25–11.25	33	21 25–23 25	12	2.21–2.41	17	11	38
1.35–1.50	17	>42.5	6	11.25–12.25	27	23.25–25.25	14	2.41–2.61	10	12	6
1.50–1.65	16			12 25–13.25	18	25.25–27.25	5	2 61–2 81	8	13	3
1.65–1.80	11			13.25–14.25	7	27 25–29.25	8	2.81–3 01	4	>13	2
1.80–1.95	7			>14.25	3	29.25–31.25	6	3.01–3.21	2		
1.95–2.10	4					>31.25	4	>3.21	7		
>2.10	5										
Total....	196										

maximum. The ratio $\frac{\text{length of root}}{\text{length of stem}}$ was calculated from the data as a measure of the stockiness of the plant.

From tables 1 and 2, it is seen that the outstanding differences between the measurements of the plants grown in agar and those grown in sand are in the amount of nitrogen fixed and in the number of nodules. While inoculated plants grown in agar fixed up to 2.1 + mgm. of nitrogen per plant, inoculated plants grown in sand fixed more than 13.0 mgm. This difference is due in part to much better opportunity for growth afforded to the plants grown in sand. The plants on agar in bottles are restricted by the poor gas exchange because of the cotton plug; carbon dioxide becomes a limiting factor in the growth and in the fixation of nitrogen (10). Possibly the number of nodules on

the plants grown in sand is greater because the root nodule organism may be able to move about more freely in the loose sand than in the compact agar medium. On agar it can penetrate the surface only at those places where the plants penetrate it. From this it might be presumed that on account of the dependency of the bacteria upon the plant to get below the agar surface, the bacteria will invade those root hairs of the plant that are near the crown and along the tap root. In sand, however, the bacteria are free to move independently of the plant. The invasion, therefore, is not necessarily restricted to the

TABLE 2

Inoculated clover plants grown in sand

NITROGEN FIXED		NODULES		LENGTH OF STEM		LENGTH OF ROOT		LENGTH OF ROOT / LENGTH OF STEM		LEAVES	
Range	Frequency	Range	Frequency	Range	Frequency	Range	Frequency	Range	Frequency	Number	Frequency
mgm.	*no.*	*no.*	*no.*	*cm.*	*no.*	*cm.*	*no.*	*no.*	*no.*	*no.*	*no.*
0.0– 1.0	10	<7 5	15	<5 25	9	<11.25	8	<1.01	10	3	2
1.0– 2.0	18	7.5–12.5	15	5 25– 6 25	4	11 25–12.25	3	1 01–1.21	16	4	15
2.0– 3.0	22	12 5–17 5	25	6 25– 7 25	4	12 25–13 25	7	1 21–1 41	29	5	36
3.0– 4.0	25	17 5–22.5	47	7 25– 8.25	20	13.25–14.25	15	1.41–1 61	43	6	41
4 0– 5.0	21	22 5–27 5	14	8.25– 9 25	18	14 25–15 25	26	1 61–1 81	39	7	32
5.0– 6 0	24	27.5–32 5	22	9 25–10.25	29	15 25–16.25	27	1 81–2 01	24	8	27
6.0– 7 0	18	32 5–37 5	6	10 25–11 25	27	16 25–17.25	18	2 01–2.21	12	9	14
7 0– 8.0	17	37.5–42.5	19	11.25–12 25	29	17.25–18 25	21	2 21–2 41	9	10	16
8 0– 9 0	14	42.5–47.5	5	12 25–13 25	20	18 25–19 25	27	2 41–2 61	7	11	5
9.0–10.0	12	47.5–52.5	21	13 25–14 25	19	19 25–20 25	20	2 61–2 81	5	12	1
10 0–11.0	5	.....	...	14 25–15 25	11	20 25–21.25	11	2 81–3 01	0	13	4
11.0–12 0	8	... ...	...	15.25–16.25	4	21.25–22 25	5	>3 01	8	14	9
12 0–13.0	6	72 5–77.5	13	16 25–17 25	4	22 25–23.25	4				
>13 0	2	.....	...	17 25–18.25	4	23.25–24 25	5				
						24 25–25.25	0				
						25.25–26 25	5				
Total....	202										

crown and to the tap root, but can take place also in the root hairs along the side roots. The difference in pCO_2 in the atmosphere available to plants is also another factor in the location of the nodules, as will be demonstrated in publication (10). This difference in the location of the nodules will be treated in greater detail under *correlation* in the next section.

STATISTICAL TREATMENT

The methods used to analyze the data given in tables 1 and 2 are discussed in detail in standard texts on statistics, e.g., Fisher (2), Rietz (8) and Yule (12).[2]

[2] Reference to the texts are made by page number whenever necessary but the formulas used are included in our discussion in order to avoid discontinuities in the treatment. The derivation and discussion of these formulas can be obtained from the references given.

Homogeneity of data

In spite of all efforts to maintain uniform conditions throughout the greenhouse, it is possible that the plants in a given series were not grown under identical conditions; this would lead to a non-homogeneous sample. In order to determine whether or not the data are homogeneous, they were tested by the method described by Fisher (2, p. 190). All of the plants grown in one bottle or jar may be considered to be subjected to the same treatment, but plants from different bottles or jars are regarded as being samples from populations with different treatments. Hence the plants in a given bottle may be looked upon as forming a *class* and the test consists essentially in the determination of the significance of correlation *within classes*. This is a type problem in the analysis of variance, the treatment of which is briefly summarized. The two following statistics are calculated from the data:

$$S_E = \sum_{1}^{kn} (x - \bar{x}_P)^2 = n\,(k-1)\,B$$

$$S_T = \mathrm{k} \sum_{1}^{n} (\bar{x}_P - \bar{x})^2 = (n-1)\,(kA + B)$$

x = individual observation (milligrams nitrogen fixed by a given plant)
$\bar{x}_P$ = mean of a class (plants in one bottle or jar)
$\bar{x}$ = mean of all observations
n = number of classes (number of bottles or jars)
k = number of observations in each class

From these two formulas, A and B may be determined, from which the *intra-class correlation coefficient* R can be estimated:

$$R = \frac{A}{A + B}$$

A may be regarded as the variance due to "causes" which observations in one class have in common, and B is the remainder of the total variance of the population. Unless R is significantly different from zero, the sample may be regarded as homogeneous. To test this the statistic z is calculated:

$$Z = 1/2\, ln \frac{V_T}{V_E} \tag{11}$$

$$V_E = \text{variance due to errors} = \sum_{1}^{kn} (x - \bar{x}_P)^2 \div n\,(k-1)$$

$$V_T = \text{variance due to treatment} = k \sum_{1}^{n} (\bar{x}_P - \bar{x})^2 \div (n-1)$$

The distribution of z has been determined for different values of n and k and tables are available which are entered with the value of z, n, and k given by the

data, and when so entered give the probability that the observed value of R is due to chance. Unless the value of this probability is less than 0.05, the observed correlation cannot be held to be significant, and the population sampled is homogeneous. The results of the tests on the homogeneity of the samples from the *milligram nitrogen* and *number of nodules* "populations" are given in table 3. Since some plants were lost in the nitrogen analyses, it was necessary, in order to have the same number for each "treatment," to use only those jars or bottles which furnished a certain minimum number of plants for nitrogen analysis. In the agar series this was taken to be 5, i.e., k equals 5; in

TABLE 3

Homogeneity test on samples of population

	DEGREES OF FREEDOM	SUM OF SQUARES (S)	MEAN SQUARE (V)	INTRA-CLASS CORRELATION (R)	Z	PROB.*
Agar series—nitrogen fixed						
Within classes	116†	17 3497§	0 1496**	0 046	0 1085	>0.05
Between classes	28‡	5 2055‖	0 1859††			
Agar series—number of nodules						
Within classes	148	13,549 0	91 55	0 048	0 1117	>0 05
Between classes	36	4,120 4	114 5			
Sand series—nitrogen fixed						
Within classes	98	978 24	9 982	0 105	0 3300	0 04
Between classes	13	251 00	19 307			
Sand series—number of nodules						
Within classes	189	54,005 9	285 7	0 216	0 4523	<0 01
Between classes	26	18,376 7	706 8			

* Probability that intraclass correlation coefficient is not different from zero, i.e., population is homogeneous.

† $= n(k-1)$; ‡ $= n-1$, § $= S_E$; ‖ $= S_T$; ** $= V_E$; †† $= V_T$.

the sand series, k equals 8. For example, in the sand series all samples from a jar that had less than 8 plants analyzed were eliminated from the test; in case more than 8 plants from a jar were analyzed, the extra samples were eliminated by chance (drawing of a card). A like system was followed in the agar series k equal to 5. Only in the case of the sand cultures did this selection eliminate a large number of samples, but even here the selected population was representative of the original population. The mean of 202 samples was 5.56 $\pm$0.22 mgm. before selection as compared with the 5.93 $\pm$0.32 (112 samples) after selection. The values of the probabilities given in table 3 indicate that only in the case of the number of nodules in sand cultures are there indications

that the populations sampled are not homogeneous. This population will be discussed later when the type of distribution is considered. There is some evidence of a slight intraclass correlation in the population of nitrogen fixed in sand cultures, but the significance of this is doubtful.

Type of distribution

The distribution of the samples of nitrogen fixed and of the number of nodules in both the agar and sand series were compared with the theoretical Gaussian (normal) curves by calculation of the first four moments from the observations. Estimates of the moments were made by the use of the usual formula (2, p. 72):

$$\mu_i = \frac{1}{n} \sum (x - \bar{x})^i$$

n = number of observations
x = individual observation
$\bar{x}$ = mean of n observations
i = 1, 2, 3 ————————

The crude moments, ν_i, were calculated from the grouped data and μ_i determined by applying the usual Sheppard's correction. The values of μ_i so estimated were used to calculate two statistics suitable for measuring departure from the normal curve, viz., α_3 and α_4 (γ_1 and γ_2 in Fisher's terminology).

$$\alpha_3 = \frac{\mu_3}{\mu_2^{3/2}} \qquad \alpha_4 = \frac{\mu_4}{\mu_2^2}$$

Since for the normal frequency curve $\alpha_3 = 0$ and $\alpha_4 = 3$, it is necessary only to determine whether or not the α's calculated from the data are significantly different from the theoretical values. This is done by comparing the difference between the observed value of α_i and its theoretical value with its standard deviation. Unless the observed difference is 3 to 4 times its standard deviation, the departure from normality cannot be held to be significant. For a normal distribution, the standard deviation of α_3 is $\sqrt{\frac{6}{n}}$ and that of α_4 is $\sqrt{\frac{24}{n}}$.

The statistics used to test the normality of the observed distribution of frequencies are given in table 4. The values obtained indicate that the distribution of nitrogen fixed by clover plants in agar or in sand is essentially normal. There is some evidence that α_3, which measures the asymmetry of the curve, is different from its theoretical value, zero, but the deviation is not marked either in sand or in agar. In both sand and agar the value of α_4, which measures the flatness of the curve, is not significantly different from its theoretical value of 3.0. On the other hand, the distribution curve of the number of nodules in both the sand and agar series is quite asymmetric toward the lower values, as indicated by the high value of α_3 for these populations. Since the differences from zero are about 6 times the corresponding standard deviations, the observed asymmetry is certainly significant. In the case of the plants grown in

TABLE 4

Statistical constants on distribution

Distribution	MOMENTS μ_1	μ_2	μ_3	μ_4	α_3	$\frac{\alpha_3 - 0.0}{\text{S.D. } \alpha_3}$	α_4	$\frac{\alpha_4 - 3.0}{\text{S.D. } \alpha_4}$
Nitrogen fixed in agar	0	8.753	10.966	209.49	0 424 ±0 175	2.42	2.734 ±0.348	0.75
Nitrogen fixed in sand	0	10.141	13 920	240 19	0 431 ±0.172	2 50	2.336 ±0.345	1.93
Nodules in agar	0	3 666	7.668	50.803	1 092 ±0.175	6.23	3.778 ±0.348	2.24
Nodules in sand	0	12 497	46 573	572.63	1.054 ±0 172	6.13	3.666 ±0.345	1.93
Nodules in sand (selected)*	0	7 022	8 558	107.80	0 460 ±0 178	2.58	2.186 ±0.356	2.29

* After elimination of those above 50 nodules.

TABLE 5

"Goodness of fit" of theoretical distribution with observed

Population \ Distribution	$\varphi(t)$			$\varphi(t) - \frac{\alpha_3}{3!}\varphi^3(t)$			$\varphi(t) - \frac{\alpha_3}{3!}\varphi^3(t) + \frac{\alpha_4 - 3}{4!}\varphi^4(t)$		
	N	X^2	Probability*	N	X^2	Probability	N	X^2	Probability
I. *Agar series:*									
Nitrogen fixation	12	11 71	0.47	11	5 00	0.92	10	4.46	0.90
Nitrogen fixation (grouping)	2	7 05	0 03	1	4.72	0 03	2	4.32	0.10
Number of nodules	7	49.09	<0 01	6	8 71	0 20	5	8.33	0.15
Number of nodules (grouping)	2	22.12	<0 01	1	6 20	0 01	2	7 37	0.03
II. *Sand series:*									
Nitrogen fixation	12	21 80	0 04	11	13 06	0 30	10	12.71	0.30
Nitrogen fixation (grouping)	4	17 62	<0 01	4	11 53	0 02	3	9.75	0.02
Number of nodules†	7	33 21	<0.01	6	22.10	<0 01	5	20 75	<0.01

* Probability that worse fit would be obtained through random sampling.

† All 13 samples containing more than 50 nodules eliminated.

the sand, part of the asymmetry is due to a second mode appearing in the distribution at about 75 nodules (see table 2). When these samples are eliminated, the value of α_3 falls to a relatively insignificant figure. In neither sand nor agar series is there any intimation of a departure from normality in regard to the flatness of the curve of distribution of nodules as measured by α_4.

The actual distributions found were compared with the theoretical normal curves and with the normal curves partially corrected for the observed asymmetry or flatness by calculating theoretical distributions from various numbers of terms in a Charlier's series (8, p. 114)

$$F(t) = \varphi(t) - \frac{\alpha_3}{3!}\varphi^3(t) + \frac{\alpha_4 - 3}{4!}\varphi^4(t) + \ldots\ldots$$

The normal curve was used as the generating function in this series, i.e., $\varphi(t)$, and by taking one, two, and three terms, the observed distributions were compared with: the theoretical normal curve, the normal curve partially corrected for evidences of asymmetry, and the normal curve partially corrected for asymmetry and flatness. The comparison was made by the χ^2 "goodness of fit" test (2, p. 75). The results of the comparison are given in table 5 and figures 1 and 2. A consideration of these figures and of table 5 is most instructive for our inquiry into the nature of the distributions of the populations. Table 5 shows that the distribution of nitrogen fixed in agar corresponds to the normal curve specified by the first two moments of the data as closely as could be expected from a sample of 200 individuals; in this case the inclusion of the correction terms merely leads to a higher probability that the observed deviations arose from chance. In the sand series, the fit of the observed data on *milligrams nitrogen fixed* to their theoretical normal curve is none too exact, as the probability that a worse fit could be obtained through sampling is only 0.04. However, the addition of the first term that corrects for asymmetry $\left[\frac{\alpha_3}{3!}\varphi^3(t)\right]$ in the series results in a satisfactory agreement between the theoretical and observed values. The distribution of the *number of nodules* in agar is characterized by an asymmetry toward the lower values, as was discussed in connection with table 5. Hence, the uncorrected normal distribution curve does not fit the data, but two terms of the proper Charlier's series describe a curve that shows fair agreement with the observations. On the other hand, the observed distribution of nodules on plants in sand does not agree with any of the three theoretical distributions calculated from the proper series. Since the selected values of these nodules were used for the test, this result is rather surprising in light of the values of α_3 and α_4 for this distribution (see table 5). A glance at the plotted frequencies (fig. 2) shows that the lack of fit is due to alternately high and low frequencies. This suggests that the original counts were not accurately determined, which resulted in an excess in the frequency of one class and a deficiency in the neighboring one. Consultation of the original data verified this hypothesis. Because of the large number and the

small size of many of the nodules in the sand series, an accurate count of the higher values is not obtained in ordinary laboratory practice. As a result of the difficulties in counting the nodules, many of the values are rounded off to the nearest 5 or even 10 units, a practice that probably accounts for the seeming irregularity in the plotted frequencies. In a much larger sample, this inaccuracy in the estimation of the nodules would be greatly eliminated by compensation; however, in the small sample taken in this experiment, it might easily lead to the observed alternate excess and deficiency in neighboring classes.

Further consideration of figures 1 and 2 leads to the suspicion that the relatively high values obtained for the probabilities that the observed frequencies

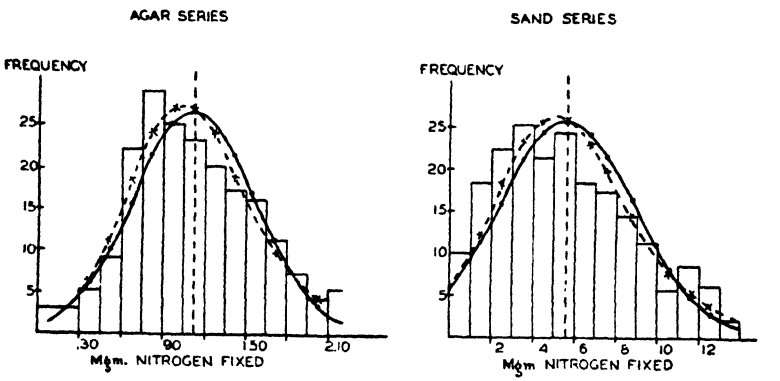

FIG. 1. COMPARISON OF THE OBSERVED DISTRIBUTION OF MILLIGRAM NITROGEN FIXED BY CLOVER WITH TWO THEORETICAL CHARLIER'S SERIES

$\varphi(t)$ is the normal curve of errors

●——● $F(t) = \phi(t)$

x———x $F(t) = \phi(t) - \frac{\alpha_3}{3!}\phi^3(t)$

$$\phi(t) = \frac{1}{\sqrt{2\pi}} e^{\frac{-t^2}{2}}$$

agree with the theoretical might be, to a certain extent, fictitious. It will be noted that to the left of the mean the observations differ from the theoretical values by an excess, and to the right by a deficiency. In no case are the differences marked, and this leads to rather high values for the probability. However, if the consecutive positive and negative residuals are combined, the resulting number of classes is decreased from 12 or 14 to about 4 or 5. When tested by this highly unfavorable method of grouping, the probabilities are reduced to about 0.02. When both methods of testing are taken into account, it can be concluded that the observations check with the theoretical curves fairly satisfactorily but not as well as is indicated by the χ^2 test without grouping.

It should be observed that the theoretical curves extend from $-\infty$ to $+\infty$, but the observed frequencies necessarily must begin at zero. The area to the left of the zero point of the theoretical curves (normal) varies from 0.5 to 4 per cent of the total; with the corrected curves these values are even less. For exact analysis, theoretical curves that begin at zero should be used for comparison, but because of the relatively small part of these curves in the negative region, the more convenient strictly normal type of curve was used in this work. Finally, table 5 shows that two terms of a Charlier's series are as efficient in describing the populations as are three terms. The values of χ^2 are lower with three terms but the loss of an additional degree of freedom, because

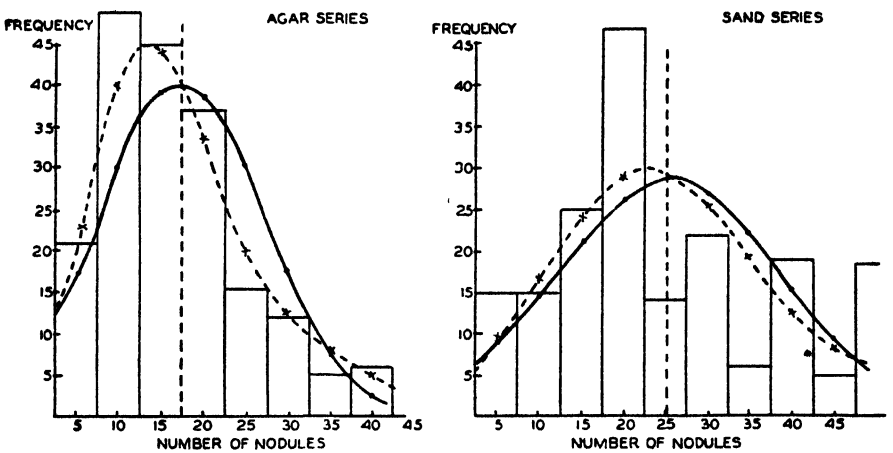

FIG. 2. COMPARISON OF THE OBSERVED DISTRIBUTION OF NUMBER OF NODULES ON CLOVER WITH TWO THEORETICAL CHARLIER'S SERIES

$\phi(t)$ is the normal curve of errors

●——● $F(t) = \phi(t)$

x———x $F(t) = \phi(t) - \frac{\alpha_3}{3!}\phi^3(t)$

$$\phi(t) = \frac{1}{\sqrt{2\pi}} e^{\frac{-t^2}{2}}$$

of the use of another moment in the specification of the theoretical curve, cancels the advantage of a lowered χ^2, and the probabilities remain much the same. This confirms the conclusion reached from the values of α_4, viz., none of the curves are decidedly peaked or flat. Since the second term of the Charlier's series corrects for asymmetry and the third term for flatness, it can be concluded that the only indication of a departure from normality in any of the curves is a slight asymmetry toward the lower values of the variables. The estimated values of the more important statistics for these distributions as well as those for the distributions of the other variables are given in table 6.

In actual practice, the data are seldom collected on *individuals* of the populations under consideration, but on samples from at least two, to as high as ten

TABLE 6

Maximum and minimum values; means, and standard deviations

	AGAR				SAND			
	Maximum	Minimum	Means	Standard deviation	Maximum	Minimum	Means	Standard deviation
Nitrogen fixed.....*mgm.*	3.24	0 16	1 13 ±0 03	0.44 ±0.02	14.27	0.33	5 56 ±0.22	3.18 ±0.16
Length of stem.....*cm.*	15 0	2 0	9 95 ±0 16	2 19 ±0.11	18 0	3 0	10 88 ±0.20	2.85 ±0.14
Length of root.....*cm.*	43 0	6 0	17.56 ±0 42	5 94 ±0 30	28 5	8 5	17.19 ±0.22	3 15 ±0.16
Number of nodules.....	51	4	17 50 ±0.68	9 57 ±0 48	100	4	28.56 ±1 24	17.68 ±0.88
Number of leaves.....	14	4	9 3 ±0 13	1 81 ±0 09	19	3	7.24 ±0.18	2.49 ±0.12
Ratio $\frac{\text{root}}{\text{stem}}$			1 82 ±0 04	0 60 ±0.03			1.71 ±0.03	0.49 ±0 02

plants. For this reason, the question in which we are particularly interested is the nature of the distribution of the *means* of a sample of n individuals from populations distributed as are those indicated in figures 1 and 2. Poincaré (7, p. 189–223) gives a demonstration which shows that even if the error functions of the individual errors are different from one another and from the normal Gaussian curve of errors, the sum of n errors will form a normal distribution, although n may be only moderate in size. The only assumptions necessary regarding the nature of the unknown error functions are that they are small, of like magnitude, and are even valued (negative errors are as likely as positive ones).[3]

[3] The same sort of a demonstration can be applied to our problem. Suppose a population forms a distribution that can be described by any function $f(t)$; what is the nature of the distribution of the *means* of samples of n individuals from this population? Without specifying the nature of $f(t)$, some idea of the distribution can be obtained by the calculation of the α's for the means of n individuals, $a_1, a_2, \ldots\ldots a_n$. The second moment of the distribution of the means (M_2) will be given by the expression:

$$M_2 = \int_{-\infty}^{\infty} \ldots\ldots \int_{-\infty}^{\infty} \frac{(a_1 + a_2 \ldots\ldots + a_n)^2}{n^2} f(a_1) \ldots\ldots f(a_n) da_1 \ldots\ldots da_n$$

Since the first moment of the *individuals* (μ_1) is taken to be zero,

i.e., $$\mu_1 = \int_{-\infty}^{\infty} a_i f(a_i)\, da_i = 0,$$

all cross product terms in the expansion of $(a_1 \ldots\ldots a_n)^2$ will disappear,

$$\therefore M_2 = \int_{-\infty}^{\infty} \frac{n a_i^2}{n^2} f(a_i)\, da_i = \frac{1}{n} \mu_2$$

Where μ_2 is the second moment of the distribution of the individuals. Likewise the third and fourth moments can be calculated:

$$M_3 = \int_{-\infty}^{\infty} \ldots\ldots \int_{-\infty}^{\infty} \frac{(a_1 + a_2 \ldots\ldots a_n)^3}{n^3} f(a_1) \ldots\ldots f(a_n)\, da_1 \ldots\ldots da_n$$

$$= \int_{-\infty}^{\infty} \frac{n a_i^3}{n^3} f(a_i)\, da_i = \frac{1}{n^2} \mu_3$$

$$M_4 = \int_{-\infty}^{\infty} \ldots\ldots \int_{-\infty}^{\infty} \frac{(a_1 + a_2 \ldots\ldots a_n)^4}{n^4} f(a_1) \ldots\ldots f(a_n)\, da_1 \ldots\ldots da_n$$

$$= \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{n a_i^4 + 3n(n-1) a_i^2 a_j^2}{n^4} f(a_i) f(a_j)\, da_i\, da_j$$

$$= \frac{\mu_4}{n^3} + \frac{3(n-1)}{n^3} \mu_2^2$$

From these moments, the values of the α's for the distributions of the means of n individuals can be calculated by the usual formulas in terms of the α's for the distributions of the individuals:

$$\bar{\alpha}_3 = \frac{M_3}{M_2^{3/2}} = \frac{\mu_3}{n^2} \cdot \frac{n^{3/2}}{\mu_2^{3/2}} = \frac{\alpha_3}{\sqrt{n}}$$

$$\bar{\alpha}_4 = \frac{M_4}{M_2^2} = \frac{\mu_4 + 3(n-1)\mu_2^2}{n^3} \cdot \frac{n^2}{\mu_2^2} = \frac{1}{n} \frac{\mu_4}{\mu_2^2} + \frac{3(n-1)}{n} = \frac{\alpha_4}{n} + \frac{3(n-1)}{n}$$

in which the bar over the α signifies that it belongs to the distribution of the means of n

Correlation coefficients

In many experiments, it is not always possible to make nitrogen determinations, nodule counts, etc. but some idea of the magnitude of these variables may be desired; this information may be approximated if the value of some other variable whose correlation with the variable not measured is known. Thus the correlation of one variable with another has practical application in the laboratory as well as theoretical interest in respect to the interrelationship between the various measurements of the growth of the plant. Both the simple and the multiple correlation coefficient among the several variables measured were calculated from the data; the results are given in table 7. The values of these estimations were tested for significance by calculation of the statistic t as described by Fisher (2, p. 159), and all were found to indicate real correlation except that between nitrogen fixed and $\frac{\text{length of root}}{\text{length of stem}}$ in the agar series. The observed correlation between the *number of nodules* and the *length of root* in the agar series is just on the limit of significance, as the probability that the observed correlation arose from random sampling is 0.05. The observed differences between the correlation coefficients for plants in agar and in sand were tested for significance by the calculation of z as described by Fisher (2, p. 163). This statistic is determined for each value of r by the formula:

$$Z = 1/2\,\{ln\,(1+r) - ln\,(1-r)\} = \log\frac{1+r}{1-r} \times 1.15$$

The difference between the z's corresponding to two values of r (r_1 and r_2) has a standard deviation equal to $\sqrt{\frac{1}{n_1-3}+\frac{1}{n_2-3}}$ (2, p. 170) where n_1 and n_2 are the number of pairs of values used in the estimation of r_1 and r_2. If the difference in the z's is less than twice its standard deviation, the observed difference in the r's is hardly significant. From the values given in column 8 of table 7, it is apparent that the observed values of corresponding simple correlation coefficients in the sand and agar series differ only in the following instances: (*a*) *nitrogen fixed* with *number of nodules*, (*b*) *nitrogen fixed* with $\frac{\text{length of roots}}{\text{length of stems}}$, and (*c*) *number of nodules* with *length of stem*. In all cases the correlation coefficient is lower in the agar series. Of the multiple correlation coefficients, it appears that the value of r for *nitrogen fixed* with *number of*

individuals. A consideration of the values of the $\bar{\alpha}$'s indicates that the f(t) may be a function that departs decidedly from the normal curve (as indicated by the value of the α's) but that *means* of even five individuals would tend to form a normal distribution. For example, if f(t) were such that the value of α_4 were only 2.0; the value of $\bar{\alpha}_4$ for means of five individuals would tend toward: $\frac{2.0}{5}+3\left(\frac{4}{5}\right)=2.8$, very close to the theoretical value of 3 for the normal curve.

TABLE 7

Summary of correlation coefficients

CORRELATION BETWEEN	AGAR SERIES			SAND SERIES			DIFF. IN z / S.D.$_z$*
	r	$\sqrt{1-r^2}$	z	r	$\sqrt{1-r^2}$	z	
Nitrogen fixed and number of nodules	0 39	0 920	0.4103	0.63	0.776	0.7384	3.25
Nitrogen fixed and length of stem	0.52	0 854	0 5747	0.63	0.776	0.7384	1.62
Nitrogen fixed and length of root	0 39	0 920	0.4103	0.25	0.968	0.2554	1.53
Nitrogen fixed and number of leaves	0 41	0 912	0 4454	0.56	0.829	0 6306	1.83
Nitrogen fixed and $\frac{\text{length of root}}{\text{length of stem}}$	−0 02	0 999	0.0203	−0.44	0 898	0.4704	4.46
Nitrogen fixed, number nodules, and length of stem	0.58	0.815	0 6595	0.71	0.704	0 8842	2.22
Nitrogen fixed, number nodules, and length of root	0 52	0.854	0.5747	0 64	0 768	0.7564	1.80
Nitrogen fixed, length stem, and length root	0.57	0.821	0 6450	0 64	0.768	0.7564	1.10
Number of nodules and length of stem	0 29	0 958	0 2983	0.56	0 829	0.6306	3.29
Number of nodules and length of root	0 14	0.990	0 1402	0.19	0.981	0.1919	0.51
Length of stem and length of root	0 34	0 940	0 3526	0.19	0.981	0.1919	1.59

* Standard deviation of the difference between z's = 0.101.

nodules and length of stem is significantly less in the agar series than in the sand series.

A consideration of the various coefficients of correlation shows that the values of r are positive except the one in the sand series between nitrogen fixed and $\frac{\text{length of root}}{\text{length of stem}}$; in this case high values of *nitrogen fixed* appear to be associated with low values of the ratio, i.e., more nitrogen is fixed by the stocky plants. Although the values of most of the estimations of the correlation coefficient are significantly different from zero, it cannot be claimed that the knowledge of the magnitude of one variable gives much information concerning

TABLE 8

Contingency tables for the type and distribution of nodules with nitrogen fixed and number of nodules (agar series)

TYPE AND LOCATION	TAPROOT		INTERMEDIATE ROOTS		TOTALS
	TR*	TL	IR	IL	
I. Nitrogen fixed *mg.*					
0.00–0 70	4 (2 7)	6 (8.1)	8 (6.4)	12 (12 8)	30
0 70–1.40	8 (9 7)	33 (29.8)	20 (23 5)	49 (47.0)	110
1.40–2.10	5 (4 6)	13 (14.1)	13 (11.1)	21 (22.2)	52
Totals	17	52	41	82	192
Test for independence	$X^2 = 3\ 30$ $n = 6$ Probability = 0.77				
II. Number of nodules					
0–20.5	11 (11 7)	39 (35.8)	20 (28 2)	62 (56 3)	132
>20.5	6 (5 3)	13 (16 2)	21 (12 8)	20 (25 7)	60
Totals	17	52	41	82	192
Test for independence	$X^2 = 10.51$ $n = 3$ Probability = 0 02				

* TR = taproot, round type; TL = taproot, long type; IR = intermediate roots, round type; IL = intermediate roots, long type; numbers in parentheses are theoretical values for cell.

the corresponding value of any other. This is indicated by the *coefficient of alienation* (8, p. 128) given in columns 3 and 6, table 7. The value of this statistic, $\sqrt{1 - r^2}$, is a measure of the improvement that can be made in the error of estimate through knowledge of the value of a correlated variable. For example, in the case of those two variables which showed the highest correlation, viz., *nitrogen fixed* and *number of nodules* in the sand series (0.63), the variability in the array of values of the number of nodules corresponding to a given value of the milligram nitrogen fixed is almost four-fifths of the average variability of *all values* of the number of nodules. Hence the correlation coefficient would aid primarily in the estimation of the *mean* number of nodules of a

large number of samples corresponding to a given value of nitrogen fixed. A comparison of the multiple with the simple correlation coefficients shows that measuring two variables gives little information with respect to a variable not estimated, in addition to that gained by the measurement of the single variable with the higher value of r. For example, the error in the estimate of the nitrogen fixed by a plant in agar is 0.44 mgm.; knowledge of the length of the stem would reduce this to $0.854 \times 0.44 = 0.376$ mgm., but the additional knowledge of the number of nodules would not help much, since in this case the error would be $0.815 \times 0.44 = 0.359$ mgm.

TABLE 9

Contingency tables for the type and distribution of nodules with nitrogen fixed and number of nodules (sand series)

TYPE AND LOCATION	TAPROOT		LATERAL ROOTS		TOTALS
	TR*	TL	LR	LL	
I. Nitrogen fixed mgm.					
0–4	7 (6 5)	15 (13 85)	5 (5 0)	49 (50 7)	76
4–8	6 (6 4)	13 (13 7)	6 (4.9)	50 (50.0)	75
>8	4 (4.1)	8 (8 5)	2 (3.1)	33 (31.3)	47
Totals	17	36	13	132	198
Test for independence	$X^2 = 0\ 98$	$n = 6$	Probability = 0 98		
II. Number of nodules					
0–20 5	10 5 (8.7)	16 (18 45)	7.5 (6.65)	68.0 (67.7)	101.5
20.5–40 5	4 5 (5 15)	14 5 (10 9)	4.5 (3.95)	36.5 (40 0)	60
>40 5	2 0 (3.15)	5.5 (6 65)	1.0 (2.4)	27.5 (24.3)	36.5
Totals	17	36	13	132	198
Test for independence	$X^2 = 4\ 33$	$n = 6$	Probability = 0.63		

* TR = taproot, round type; TL = taproot, long type; LR = lateral roots, round type; LL = lateral roots, long type; numbers in parentheses are theoretical values for cell.

For the estimation of association between variables of which one or more are capable only of qualitative measurement, the use of the contingency table is of value. In the analysis of the data on the number of nodules on plants in sand and in agar two striking differences were encountered, viz., much higher numbers of nodules in the sand series, as well as the appearance of a second mode in the frequencies of the nodules. It was believed that these differences might be associated with the location of the nodules on the roots. The type and location of the nodules was estimated in a qualitative manner by classifying the nodules on each plant into: (*a*) round or long type (*b*) predominately located on the tap, intermediate, or lateral roots. It is realized that a classification of this type can be at best only approximate, since a great deal depends on the

personal bias of the observer, but in spite of these limitations, the use of this description of the location and type of nodules has proved of value in many experiments. Examination of the original data showed that the invasion of the lateral roots was almost negligible on the plants grown in agar, but that this type of invasion ranks very high on plants grown in sand. For example, in agar, 69 of the 196 plants examined showed nodules primarily on the taproot, 123 on the intermediate roots, and only 4 on the lateral roots. In sand, however, 53 of the 202 plants had nodules predominately on the taproot, 4 on the intermediate roots, and 145 on the lateral roots. It seems likely that the difference between the numbers of nodules is connected with the differences in the location. In order to determine whether the type and location of the nodules were associated with nitrogen fixed or with the number of nodules the contingency tables 8 and 9 were prepared and tested for independence by the X^2 test (2, p. 82). Whenever the location of the nodules on a plant was not definitely in one class, it was divided into 0.5 units for classification as shown in table 9. In both the sand and agar series, the values of the probabilities found are very high that there is complete independence between the *type and location of the nodules* and *nitrogen fixed*; this is likewise true for the sand series between the *type and location of nodules* and *the number of nodules*. However in the agar series, there is evidence that there is some association between the *type and location of nodules* and *the number of nodules*. Upon examining the individual values of the X^2 contributed by the various cells in the table, it appears that the indicated association is due to an excess of the round type of nodule on the intermediate roots of those plants having more than 20 5 nodules. In order to investigate this further and to determine whether there is any masking of association between two of the variables in a large table of this type, the data given in tables 8 and 9 were reassembled in four-fold and six-fold types of contingency tables and the following variables tested for association (12, p. 378):

Location of nodules with number of nodules.
Type of nodules with number of nodules.
Location of nodules with type of nodules.
Location of nodules with nitrogen fixed.
Type of nodule with nitrogen fixed

In both the sand and agar series, complete independence of the foregoing pairs of variables was found except that in the agar series the long type of nodules was associated with the lower number of nodules, and in the sand series, there was an excess of the round type of nodules on the taproot.

SUMMARY

Inoculated clover plants were grown on agar in cotton-plugged bottles and on sand in open jars in the greenhouse and the following measurements taken: milligrams nitrogen fixed; number, type, and location of the nodules; length of stems and roots; and number of leaves.

From these data, a statistical analysis was made which included the following tests and calculations: homogeneity of the data on milligrams nitrogen fixed and on the number of nodules per plant; distribution curves of the nitrogen fixed and the number of nodules; simple and multiple correlation coefficients among the variables capable of quantitative estimation; and association tests among those variables which admit of only qualitative specification. The statistical methods used are discussed in detail.

The statistical tests of the data showed, that under the conditions of the described experiments, the populations sampled, for example, milligrams nitrogen fixed, are homogeneous with the exception of the number of nodules in the sand series. However, even in this case, the non-homogeneity is not marked. Likewise, the distribution curves of the variables examined are essentially normal except that of the number of nodules in the sand series. This observed departure from normality appears to be due to inaccuracies in counting the nodules rather than to any gross abnormalities in the type of distribution. A mathematical demonstration is given that shows normal statistics can be applied with confidence to the populations investigated (milligrams nitrogen fixed and number of nodules) if a few plants are combined for a measurement.

The simple correlation coefficients indicate that no two of the variables measured are very highly correlated with each other. In the sand series the greatest correlation was observed between the number of nodules and the milligrams of nitrogen fixed (0.63) and the length of stem and milligrams of nitrogen fixed (0.63). In agar, the highest correlation coefficient was between the length of the stem and milligrams nitrogen fixed (0.52); the correlation between the number of nodules and milligrams nitrogen fixed was only 0.39. Multiple correlation coefficients in which two variables were correlated with milligrams nitrogen fixed were only slightly better than the higher of the two simple correlation coefficients between milligrams nitrogen fixed and these variables.

The following pairs of variables were tested for association in both the agar and sand series: location and type of nodules with number of nodules and with milligrams nitrogen fixed; location of nodules with type of nodules. Association was indicated only between type of nodules and number of nodules in agar and between type of nodules and location of nodules in sand.

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RELATION BETWEEN CARBON DIOXIDE AND ELEMENTAL NITROGEN ASSIMILATION IN LEGUMINOUS PLANTS[1]

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Two of the most important chemical reactions in nature are the assimilation of carbon by higher plants and the assimilation of elemental nitrogen by microörganisms. It is a commentary upon the economy of nature that these two life-supporting syntheses should occur simultaneously in one family, viz., the *Leguminosae*. Both processes have been studied independently of one another, but little work has been done with the view of determining the interrelation between them. Such studies might be made in two ways: by a comparison of the carbohydrate and nitrogen metabolism of plants fixing nitrogen with others grown under similar conditions but supplied with combined nitrogen; by modifying one process, e.g., photosynthesis, and observing the effect on the other. During the past year studies have been started on the problem of the relationship between carbon assimilation and nitrogen fixation in *Leguminosae* by use of the second method of attack; the results are given in this report.

Modification of the carbon assimilation process might be accomplished in a variety of ways: change of temperature, pressure of CO_2, intensity or quality of light. Nitrogen fixation may be influenced: by the addition of a source of combined nitrogen, e.g., nitrates, by the use of different strains of rhizobia, and by other factors. Since many of the factors concerned with nitrogen fixation are not known, it appeared advisable to limit the first experiments to changing the photosynthetic activities of the plant rather than attempting to control the nitrogen fixation. Later, experiments will be made in which both carbon assimilation and nitrogen fixation will be modified. The variable factor controlling photosynthesis that can be most easily regulated in greenhouse experiments is the partial pressure of CO_2 in the atmosphere supplied to the plants; therefore, this variable was selected for study. Specifically, plants were supplied with atmospheres containing different partial presssures of CO_2 and the effects on nodule formation, nitrogen fixation, dry weight, and the general anatomy of the plant were determined.

It is not feasible in this paper to attempt a complete review of the literature concerned with the effect of increased CO_2 supply on the growth of plants. The monographs of Reinau (15), Fischer (6), Bornemann (3, 4), and Lundegardh (12) contain excellent historical reviews of this subject; and less detailed accounts are given in Spoehr (19), Stiles (20), and Niklas, Scharrer, and Strobel (13). However, it will be advantageous to consider briefly certain points which deal with the historical and practical aspects of the subject, before the more detailed review of the literature concerned with the leguminous species is made.

HISTORICAL

In 1804, de Saussure (18), grew young pea plants in atmospheres containing 8 to 100 per cent CO_2, and exposed to bright or diffuse sunlight. In the diffuse light, all concentrations of

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CO_2 were detrimental to the plant; but in the full sunlight, the plants receiving 8 per cent CO_2 showed better growth than the controls grown in ordinary air. Albrecht Thaer (21), in 1837, emphasized that the value of the use of organic manures was partially due to the CO_2 liberated by their decomposition.

Krueseler (10) in 1885, grew plants in mixtures of carbon dioxide and air ranging from 0.03 to 12 per cent. He used an arc light of 1,000 candle power in order to control the intensity of light during the experiment; this light source was placed 30 to 45 cm. from the plants and was separated from them by a water screen. Kruessler found that the assimilation of CO_2 was dependent on the partial pressure of CO_2 in the atmosphere. As the pCO_2 was raised from 0.03 to 1.0 per cent the increase in assimilation was quite rapid but became less rapid from 1 to 6 per cent; finally an increase in the pCO_2 above 6 per cent had no further effect on the assimilation.

Demoussy (5) grew a number of ornamental plants in closed glass boxes and supplied them with an atmosphere which contained about 0.2 per cent CO_2; the plants receiving CO_2 had dry weights of 2 to 5 times those of the controls which were supplied with ordinary air. Since 1910, a great deal of work has been done in Europe in regard to the practical aspect of addition of CO_2 to plants in greenhouse and field; the feasibility and technique of the so-called "Kohlensäuredüngung" has engaged the efforts of a large number of investigators, notably Reinau, Lundegardh, Bornemann, Fischer, Klein, and Riedel. The contributions of these workers will be discussed in subsequent sections.

Sources of CO_2

The pioneer work of de Saussure (18), Kruessler (10), and others demonstrated that the dry weights of plants could be materially increased by the addition of CO_2 to the atmosphere. The investigations of the later workers have dealt with practical methods for increasing the supply of CO_2 and more detailed analysis of the effects. Before considering the results of the latter studies, it might be of interest to review some of the proposed methods for increasing pCO_2 in atmospheres supplied to plants. The methods are conveniently divided into the inorganic and the organic, or more properly, biological. The obvious example of the first type is the use of compressed CO_2 in tanks or by the liberation of CO_2 from $CaCO_3$ with acids. These methods are certainly the most convenient for experimental work and are used almost exclusively in theoretical studies. In addition, some experiments have been made in both greenhouse and field which indicate that the judicious use of CO_2 in cylinders in these places might be economical.

A second type of the inorganic method is CO_2 production by burning coke, charcoal, or alcohol. Reinau has patented a burner called the *OCO Dunggasspender* which burns a standard coke briquette; this produces a known quantity of CO_2, so that in a closed space, e.g., a greenhouse, the pCO_2 can be regulated to a certain extent. The burner is so constructed that it produces little heat and no poisonous gases; hence it can be used without purification apparatus. Riedel (16) has patented a larger type of CO_2 producer from which the gas is piped to greenhouse or field; he has also purified gas from blast furnaces and used it as a source of CO_2 in greenhouse and field experiments. Niklas, Scharrer, and Strobel (13) describe a patented mixture of the composition: peat, 50 per cent; wood charcoal, 45 per cent; MnO_2, 5 per cent; this apparently benefited certain plants when applied at the rate of 350 to 1,070 pounds per acre.

Turning to the organic or biological methods, we find that one of the favorite proposals is to add peat, lignite, or other sources of carbon to the soil often accompanied by inoculation with bacteria which are known to oxidize carbon. In Germany during the war, a product called *Guanol*, which apparently is a mixture of peat with spent fermentation liquor, was used extensively. The effects of this fertilizer on crop yields were greater than the mineral content warranted and the additional action was ascribed to a slow fermentation which furnished a source of CO_2 to the plants. Other commercial products of this nature include ***Humit***,

Bayerischer.—Kohlensäuredünger, and *Humuskohle*, In England, Bottomley suggested the use of a similar fertilizer called *Humogen* which was peat inoculated with bacteria; the results with this product are conflicting.

Reinau describes a greenhouse which was supplied with CO_2 from the fermentation of manure; water was sprayed over the manure and after the ammonia was scrubbed out, the fermentation gases were led over the plants. In this connection the CO_2 from the respiration of soil bacteria is probably very important in the natural CO_2 fertilization. This must be expecially true when the soil is high in organic matter, i.e., after the addition of stable or green manures. Lundegardh considers this source of CO_2 as one of the most important from a practical point of view, and he is inclined to the belief that it is the sole feasible, economical way of increasing the CO_2 in the atmosphere over a field. He has shown that over an average oat field, two-thirds of the soil respiration is due to soil bacteria and one-third to the root system of the plants. The CO_2 production is at a maximum in those fields which are high in organic matter, well aerated, and supplied with ample mineral salts and moisture.

The respiration of plant roots also can not be disregarded as a means of increasing the pCO_2 in the atmosphere about the plants. The rate of respiration is on the average only about one-seventh that of photosynthesis, but since plants respire CO_2 24 hours and assimilate only 6 hours a day, it follows that about one-half of the CO_2 assimilated during a day is respired and once more takes its place in the carbon dioxide cycle (12).

Results in practice

An adequate review of all the results obtained in greenhouse and field experiments is neither possible nor desirable for this paper, but a few of the important experiments will be discussed briefly. Reinau (16) furnished his *OCO Dunggasspender* to a large number of gardners and horticulturists, and requested testimonials in regard to its value for greenhouse and hotbed use. Even after discounting the lack of control in these experiments, the results appear to be overwhelmingly in favor of CO_2 fertilization. Bornemann (4) describes experiments which indicate that the entrance of the different growing forms, viz., vegetative, blooming, fruiting, and storage of reserves are all hastened by supplying plants with CO_2. Harder, Keppler, and Reusz (8) investigated the effect of added CO_2 and artificial light on plants grown in the greenhouse during the winter. With several species of flowers, they demonstrated that excellent and early blooms could be induced by the light plus CO_2; less striking results were obtained with light or CO_2 alone. With strawberries, ripe fruit was produced during the winter months only when both CO_2 and artificial light were used. Fischer (6) has also demonstrated that earlier blooming and fruiting can be induced in a number of plants by increasing the CO_2 in the atmosphere in which they are grown; there is also some evidence that resistance to diseases is increased in plants supplied with increased CO_2.

In field experiments the results have been encouraging in spite of numerous technical difficulties. Riedel (16, 17) purified gas from blast furnaces, piped it into fields growing barley, lupines, and potatoes, and obtained increase of 50 to 190 per cent in the dry weights of the plants so treated over those of the controls. Larger leaf surface, earlier blooming, and a greater yield of fruit were typical differences noted in the plants receiving CO_2. Bornemann (3) used CO_2 from cylinders in field experiments and found increases of 25 to 210 per cent in the yield when compared with the control plants. He also used *Guanol* as a source of CO_2 in field experiments and obtained very remarkable increases in the yield of peas, turnips, mustard, and strawberries. However, with Bottomley's *Humogen*, the results were negative. Niklas et al. (13) used a patented peat-charcoal MnO_2 mixture as a source of CO_2 and obtained increases in 7 out of 12 trials. Lundegardh (12) criticizes the results of Fischer and Bornemann on the ground of the lack of proper controls made necessary through differences in the fertility of the plots used. He also objects to the use of high concentration of CO_2 in field trials, arguing that this is not feasible in actual practice. In his own experiments, the CO_2 was supplied at about the same rate at which it would be liberated from the soil; for example, in one trial,

10,000 liters of CO_2 per 100 sq. m. were supplied to turnips during the growing season at the rate of 0.4 cc. per minute. As much as 42 per cent of the CO_2 added was recovered in the crop.

Effect of CO_2 on leguminous plants

In reference to our own experiments, the effects of added CO_2 on leguminous plants are of special interest, therefore the available data will be considered in greater detail than was possible in the foregoing general section. In the reports made to Reinau (15) on the efficacy of his *Dunggasspender*, practical gardeners state that leguminous plants gave increased yields of 50 to 100 per cent when supplied with CO_2. Riedel found that beans grown with additional CO_2 had a dry weight of 1.5 that of the controls with 5.6 times as many blooms and 5 times as many nodules. Lundegardh observes that leguminous plants are especially "thankful" for CO_2 fertilization; in a greenhouse experiment, he found that by increasing the concentration of CO_2 51 per cent, an increase in the yield of beans of 112 per cent was obtained. In similar experiments in which the concentration of CO_2 was varied from 0.03 to 0.08 per cent, it was observed that the response of beans to an increase in the pCO_2 was much greater than was that of cucumbers and tomatoes. Lundegardh also reports that beans grown in an atmosphere containing 0.08 per cent CO_2 had 10 per cent more chlorophyll in the leaves than did beans grown in air.

Cummings and Jones (4a) report that both beans and peas when treated with CO_2 gave higher yields than did the untreated controls; the gassed samples are lower in protein, i.e., more carbohydrate material is formed.

Niklas et al. (13) grew red clover in soil to which a patented peat mixture was added and found slight losses in the dry weights when compared with the controls, whereas gains were noted with barley, oats, potatoes, and field beans. Vouk (23), likewise, could not obtain any evidence of beneficial action when lignite or peat was added to soil used to grow peas, vetch, and beans, although radishes, *Tradescantia*, lettuce, and tomatoes were markedly benefited. The higher concentrations of the lignite or peat were toxic to the leguminous plants although with vetch an increase in the number of nodules was noted; however, with beans a decrease of nodules was observed. Vouk was inclined to the belief that the effect of the lignite or peat was dependent on its nitrogen content. Lieske (11) disputes this and cites experiments of his own in which a "rough lignite" was treated with ammonium salts and added in very small quantities to the soil (1 to 50 gm. per 30 kgm. of sand plus soil). In all cases a beneficial action was noted which was independent of the nitrogen added. He thinks Vouk's evidences of toxicity were due to large quantities of lignite added (10 to 100 parts per 100 parts soil); in Lieske's experiment both bush-bean and lupines gave increased yields when treated with the lignite preparation. However, Lieske attributes the activity of the lignite to its colloidal nature, which aids the plant in its nutrition by modification of the permeability of the plasma membrane; he believes other effects, e.g., CO_2 production, are secondary.

Arthur, Guthrie, and Newell (2) found that the addition of CO_2 and the use of artificial light were very effective in hastening the growth of soybeans and clover. The clover was grown from seed to flower in 38 days provided 18 hours of light were used in conjunction with 0.3 per cent CO_2. With CO_2 and added light the dry weight of the plants was 2 to 8 times that of the controls; most of this increase was in the carbohydrate fractions as the ratio $\frac{\text{carbohydrate}}{\text{nitrogen}}$ was about 4 for the control plants and 12 for the treated. Similar results were obtained with soybeans. In neither case was there any mention of nodules, and it appears that the plants were furnished combined nitrogen.

In general, the results indicate that the *Leguminosae* are decidedly responsive to an addition of CO_2; the negative results reported are likely due to the method used to supply this gas. Reinau (14) adds other evidence that sheds light on the importance of increased CO_2 supply to leguminous plants. He observed that the respiration of soil bearing clover or seradella is

much higher than is that of soil cropped to rye or mustard; also the respiration of the soil between rows of clover is only about 50 per cent of that near the plants. If the tops of the legumes are removed, however, the soil respiration decreases 33 to 50 per cent, whereas with non-legumes the decrease is only 5 to 10 per cent. He also observed that the CO_2 content of the air near the soil of fields planted with leguminous species is much higher than is that of the air near the tops of the plants. Reinau interprets these data as indicative of a high rate of respiration by the bacteria in the nodules of the plants; this respiration liberates sufficient CO_2 to increase the pCO_2 in the atmosphere surrounding the lower leaves and these leaves are thus able to assimilate CO_2 in spite of the lower light intensity available to them. It appears that a miniature CO_2 cycle characterizes the development of legumes: CO_2 is assimilated by the upper leaves; the carbohydrate formed is partially respired by the bacteria in the nodules; and the CO_2 liberated is resynthesized by the lower leaves.

In this connection, Kostytschew (9) has shown that the rate of CO_2 assimilation per unit leaf area is approximately the same for most species except the *Leguminosae*, which have much higher rates. He believes that this difference is related to the nitrogen supply of the plants but his evidence is not too convincing. It is of interest that *Alnus*, which also forms nodules, has a rate of assimilation about equivalent to that of the non-leguminous species. Connected with this high rate of assimilation and intensifying it in actual practice is the large leaf surface per unit of soil occupied by legumes as compared with other species. Bornemann (3) cites data which show that the leaf surface per square meter of soil for alfalfa and clover is much higher than for rye, oats, barley, and wheat.

In conclusion, it is to be noted that the experiments dealing with the effect of CO_2 on the growth of leguminous plants have in general been made from the point of view of CO_2 fertilization, and little attention has been given to the nitrogen fixation aspect. In most of the papers reviewed, it was not even stated whether the plants were inoculated or supplied with combined nitrogen. For these reasons, it is believed that a more detailed study of the effects of the addition of CO_2 to legumes is desirable and that such studies should yield valuable information in regard to the nitrogen fixation process.

THE EFFECT OF CO_2 ON NITROGEN FIXATION BY CLOVER AND ALFALFA

The experiments described in this section were made with clover and alfalfa plants grown in bottles in the greenhouse under various partial pressures of CO_2. The technique for growing the plants has been given in detail in another publication (24). A few methods used in the chemical analyses of the plants will be described in this paper.

Methods

Dry weight. The plants were washed from the sand or removed from the agar with large forceps and placed on filter paper in a 37°C. incubator which was provided with a large fan to keep the air circulated. After 48 hours, the plants were transferred to a ¼-pint milk bottle, capped, and kept in the incubator until ready for analysis. The dry weight, to the nearest 5 mgm., was determined on an analytical balance.

Total nitrogen. Total nitrogen in the absence of nitrates was determined by the official Kjeldahl method; in the presence of nitrates the salicylic acid modification was used.

Sand. Great difficulty was experienced in removing the sand from the roots, in spite of long washing with tap water. Since the percentage of nitrogen in

the plant is used as a criterion of the effect of the added CO_2, it is necessary that the dry weight be fairly accurate; this is impossible unless the sand is estimated in some manner. The following method was developed for this purpose and proved very satisfactory. The plants are removed from the sand, the roots washed well in flowing tap water, then soaked in 20 per cent NaCl solution for 10 minutes, followed by a second washing in tap water. This procedure removes most, but not all of the adhering sand. The plants are dried, weighed, and digested for the nitrogen determination; the digestion allows the sand present to collect at the bottom of the Kjeldahl flash, where after dilution with 250 cc. distilled water, it can be estimated by a comparison with "sand standards." These standards are made by adding a known weight of sand to about 1 gm. of sand-free plant material, digesting with H_2SO_4, etc., and finally diluting with 250 cc. distilled water. The standards used contained 25, 50, 100, 150, 250, and 300 mgm. of sand; with these standards, the sand in an unknown can be estimated with a maximum error of 10 to 15 per cent. Since the dry weight of plant material is about 10 to 30 times that of the sand present, the maximum error in the corrected dry weight of the plants is about 1 per cent. For example, with careful washing, the sand on the roots can be reduced to less than 150 mgm. per 10 plants; this quantity can be estimated by the method within 15 to 20 mgm. The dry weight of 10 plants is 1 to 1.5 gm., hence the error in the estimated weight becomes negligible. The method was tested by determining the sand in a number of unknowns which contained 10 to 275 mgm. of sand. The estimations were made by two individuals independently of each other, with results that checked each other and the true values within the indicated limits of accuracy.

EXPERIMENTAL

Experiment 1

As a preliminary experiment, red clover was grown on sand in 32-ounce bottles, supplied with an atmosphere containing about 0.5 per cent CO_2 and the nitrogen fixed compared with control plants grown in bottles closed with a cotton plug. Six plants were placed in each bottle and both sets were inoculated with *Rh. trifolii* 209, a strain that is known to fix considerable quantities of nitrogen in association with the host plant. The technique for preparation of the atmospheres was not developed at this time, and as a result the percentage of CO_2 in the mixture varied considerably. The plants were placed in the greenhouse on August 14, 1930; 14 days later those to receive CO_2 were connected to the manifolds. The plants were analyzed 72 days after planting. At this time those receiving CO_2 had completely filled the bottles, whereas the inoculated controls in the cotton plugged bottles were very much smaller. The nitrogen fixed per 10 plants by those supplied with CO_2 was 5.7 mgm. (11 samples) as compared with 1.2 mgm. (7 samples) fixed by the plants grown under a cotton plug.

The experiment was repeated except that the plants were grown on agar.

The cultures were planted November 8, 1930, but the growth was slow; on December 1, artificial lighting was started and there was noticed an immediate improvement in the plants, especially those receiving extra CO_2. The plants were analyzed after 58 days; at this time, those treated with CO_2 were tall, sturdy, and showed some signs of carbohydrate excess (red stems), whereas those grown in the cotton plugged bottles were pale and slender. The analyses are given in table 1, experiment 1. The statistical constants were calculated by the usual formulas and serve to give some idea of the variation among the individual samples. The data show conclusively that supplying clover plants grown in agar with CO_2 results in a pronounced increase in the quantity of nitrogen fixed; however, there is a greater increase in the dry weight of the plant so that the percentage of nitrogen in the plant actually decreases.

Experiment 2

The preliminary experiments indicated that the nitrogen fixation process in clover was partially dependent on the photosynthetic activities of the plants, but further experiments were believed necessary to verify this conclusion. Two questions required immediate answers before the problem could be further investigated, viz., (*a*) would the beneficial effects of the CO_2 be observed during seasons in which light intensity was not limiting photosynthesis by the plants; (*b*) how much of the benefit noted could be ascribed to aeration *per se*, independent of the added CO_2? In order to investigate these questions, experiments were made during the spring and summer of 1931 in which additional controls were included; these controls were supplied with air containing 0.03 per cent CO_2 at the same rate as were the cultures aerated with the 0.5 per cent CO_2 mixture. Plants on agar were set up May 21, 1931, inoculated with *Rh. trifolii* 209 and placed in the greenhouse. These were connected to the manifolds 14 days later; the growth was so rapid in spite of a heat wave during June, that 46 days after planting the plants were ready for analyses.

The plants which received CO_2 were dark green with red stems (carbohydrate excess), thrifty, sturdy, and inclined to be pubescent. These plants had a large number of long finger-like nodules on, or near, the taproot and also a number of small, round scattered nodules. The appearance of the nodules was very striking; many were abnormally large and very pink. There did not appear to be any correlation between the number of nodules and the appearance of the plants, except that those plants which had the most numerous and largest nodules did not show so much evidence of carbohydrate excess. The plants receiving air were light green, smooth, and spindly, with no signs of carbohydrate excess. The nodules were long and located for the most part on the taproot near the crown. These plants were no better than the plants grown in bottles closed with cotton plugs; this was very likely because of the rapid growth of the plants receiving CO_2, which made it necessary to analyze all the plants before any difference in the two sets of controls could manifest itself.

The results of the analyses are given in table 1, experiment 2, agar series. The average length of the tops and roots gives some idea of the relative stockiness of the plant; the results show that the plants receiving CO_2 were much

larger than the others, with a short, heavy root system. The increase in the number of nodules was 3 to 4 fold when CO_2 was supplied. As noted in experiment 1, there was an enormous increase in the nitrogen fixed but, since even this increase did not keep pace with the dry weight accumulation, the percentage of nitrogen in the plants receiving CO_2 showed a significant decrease.

At about the same time as the agar series was set up, the experiment was duplicated by a series in which sand plus modified Crone's solution was used as a substrate for the plant. The growth of the plants receiving CO_2 was again so rapid that it was necessary to make analyses at the end of 34 days;

TABLE 1

The effect of additional CO_2 on the growth and nitrogen fixation by clover plants

Experiments 1 and 2

TREATMENT	NUMBER OF SAMPLES	LENGTH OF TOPS	LENGTH OF ROOTS	NUMBER OF NODULES	DRY WEIGHT*	NITROGEN†	
						Total	Per cent
		cm.	*cm.*		*mgm.*	*mgm.*	
Experiment 1:							
Agar series—58 days:							
Air plus CO_2	9	...	.	.. ..	502 ±17	12 60 ±0.40	2 49 ±0 04
Cotton plug	9	...	....	... .	103 ± 4	3.80 ±0 20	3 69 ±0 14
Experiment 2:							
Agar series—46 days:							
Air plus CO_2	8	14.0	7.0	290 ±18	879 ±72	21 30 ±1.70	2.43 ±0 13
Air	4	8 2	7.0	80 ±16	153 ±20	5 25 ±0.92	3.43 ±0.20
Cotton plug	4	9.6	6.8	100 ± 5	186 ± 4	6.46 ±0 25	3.47 ±0 11
Sand series—34 days:							
Air plus CO_2	5	13.0	6.5	83 ±13	536 ±87	14.80 ±2.60	2.76 ±0.06
Air	5	8.4	7 0	34 ± 5	120 ±13	3 41 ±0 26	2 84 ±0 11
Cotton plug	3	8 8	7 0	35 ± 4	121 ± 2	3.64 ±0 20	3 01 ±0 09

* All figures in the tables are for 10 plants.

† These data are total nitrogen in the plants; uninoculated controls in agar contained 1.8 mgm. nitrogen; in sand, 2.0 mgm. of nitrogen.

and, as in the agar series, the plants receiving air alone did no better than those grown under cotton plugs.

The plants supplied with additional CO_2 were deep green, sturdy, and showed no signs of carbohydrate excess. The nodules on these plants were very large but not nearly so numerous as in the agar series; their color was pink to gray, and they were located on the taproots. Some of the largest plants had only 5 to 6 nodules per plant, but these nodules were abnormally large. The plants receiving air and those grown under a cotton plug were smaller, paler in color, and much less stocky than the CO_2 treated samples. They possessed very few nodules, but those present were fairly large.

The analyses are given in table 1, experiment 2, sand series. In general, the results confirm those obtained with the plants grown on agar with one

outstanding exception, viz., there is no significant difference in the percentage of nitrogen in the plants.

Experiment 3

The results of the first two experiments definitely established that the quantity of nitrogen fixed by clover can be greatly increased if the photosynthetic activities of the plant are accelerated. In experiments 1 and 2, the CO_2 was added to the air by regulating the flow of bubbles through a tell-tale fitted with a 1-mm. capillary tube; since this method of control was not very exact, the CO_2 content of the air fluctuated greatly. Further experiments were deemed necessary to analyze in greater detail the effects of CO_2 on the nitrogen fixation process under more controlled conditions. Since in the first experiment, the CO_2 content of the air was the same for all plants receiving the air plus CO_2 mixture, it was decided to investigate the effect of different pCO_2 in the atmospheres supplied the plants.

On July 21, 1931, red clover was planted on sand in 64-ounce bottles; the larger bottles were used in this and subsequent experiments in order to allow more room for the growth of the plants. Eight hundred grams of sand plus 135 cc. of modified Crone's solution were placed in each bottle, in which 10 seedlings were planted and then inoculated with *Rh. trifolii* 209. Eight to ten of these cultures were connected to manifolds which supplied atmospheres of the following CO_2 content; air (0.03), 0.1, 0.2, 0.4, and 0.8 per cent. In addition to the usual cotton plugged controls, which represent plants growing in an atmosphere with a CO_2 content less than 0.03 per cent, a few bottles were left open to the air. It has been long observed that plants grown in a closed system never develop as well as plants grown in air; the factors concerned in this are probably differences in the quality and intensity of the light received and differences in transpiration and respiration. However, the appearance of some of the plants receiving CO_2 in the preliminary experiments compared very favorably with plants grown in open pots. In this experiment, a direct comparison between plants grown in the open with those in a closed system was made possible by the inclusion of the "open" controls.

Periodic analyses showed that the percentages of CO_2 in the gas mixtures remained within 5 to 10 per cent of the values desired, e.g., the CO_2 in the atmosphere that was supposed to contain 0.2 per cent of this gas fluctuated between 0.18 and 0.22 per cent. Analyses of the air supplied the plants and that in the greenhouse indicated that the CO_2 content varied between 0.025 and 0.035 per cent. The plant cultures were attached to the manifolds immediately after planting and were supplied with the various mixtures at the rate of 80 cc. per minute per bottle. The weather was excellent during the period of growth with few cloudy days and with no extended periods of intense heat. The plants were removed for analysis 41 days after planting.

0.8 per cent CO_2. These plants were dark green, thrifty, and pubescent. There was little sign of carbohydrate excess and the leaves were very large. There were many nodules on the

plants both large and small; the long type was clustered near the crown on the taproot with the small, round variety scattered among the secondary roots. The nodule distribution was characteristic of plants inoculated with poor strains of *Rh. trifolii*, Allen and Baldwin (1), indicating that the addition of CO_2 had changed the usual distribution of the nodules.

0.4 per cent CO_2. The plants receiving 0.4 per cent CO_2 were little different in appearance from those supplied with 0.8 per cent of this gas. Several showed more carbohydrate excess, but the luxuriance of development was about the same. The distribution and size of the nodules were practically the same as in the 0.8 per cent series except there was less tendency for invasion of the secondary roots.

0.2 per cent CO_2. The plants in this series were smaller and had fewer leaves than those receiving higher percentages of CO_2. The majority of the nodules were long and finger-like and were on the taproot near the crown.

0.1 per cent CO_2. These plants were similar to the 0.2 per cent series except they were not quite so large and had fewer leaves. The nodules were medium to large in size and were found on the taproot.

Air (0.03 per cent CO_2). The plants supplied with air were healthy and thrifty in appearance but not nearly so large as the plants receiving additional CO_2. Especially noticeable were the smaller leaves on these plants when compared with the other series. The size and distribution of the nodules differed little from the 0.1 per cent series and were typical of plants inoculated with a good strain of the organism.

Cotton plugs. In this series the plants were pale green, much smaller than any of the others, and spindly. The nodules were small and were found on the taproot.

Open to air. Growth of the plants in the bottles open to the air was excellent but no better than in the closed bottles receiving increased CO_2; the plants were sturdy, healthy, and dark green. The nodules were predominately on the taproot with some scattered on the secondary roots.

The data on the analysis of the plants are summarized in table 2, experiment 3. The increased growth of the plants receiving CO_2 is shown by the data on the length of tops and roots. There is a noticeable difference in the size of the plants supplied with air and those which received 0.1 per cent CO_2, but additional CO_2 did not appear to cause a corresponding increase in size. This is possibly because the closed container limits the height which a plant can reach. The number of nodules per 10 plants was doubled as the CO_2 content increased from 0.03 to 0.1 per cent and was increased 3-fold in the higher concentrations of CO_2. The response to increased CO_2 in the atmosphere is strikingly shown by the data for dry weight and nitrogen content of the plants. As the CO_2 supplied was increased, both the dry weight and total nitrogen of the plants increased, but there was a large variation in the response of the individual plants of a given series as indicated by the large standard deviation of the means.

Obviously, there are striking differences between the plants receiving CO_2 and those supplied with air alone, but it is difficult to decide whether or not the differences noted among the four series treated with different pCO_2 are significant. In order to determine whether the observed differences are real or only apparent, the results from the series receiving additional CO_2 were subjected to an analysis of variance (7, p. 196). The statistical test consists essentially in combining populations receiving different treatments and examining the resultant population for homogeneity. If the population is non-

homogeneous when tested by the appropriate method (z-test 7, p. 194), the observed differences between various treatments are probably significant. The results of this analysis showed that in every case, the treatments had given rise to a real difference in all of the variables measured. The differences in the means of the variables (dry weight, number of nodules, etc.) were further tested in pairs for significance by the modified "Student" method (7, p. 107).

The results are summarized in table 3 in the form of the probability that the difference noted between any two means could have arisen from random sampling; unless this probability is 0.05 or less, the difference cannot be held

TABLE 2

The effect of the pCO_2 on the growth and nitrogen fixation by clover plants

Experiments 3 and 4

TREATMENT	NUMBER OF SAMPLES	LENGTH OF TOPS	LENGTH OF ROOTS	NUMBER OF NODULES	DRY WEIGHT	NITROGEN	
						Total	Per cent
		cm.	*cm.*		*mgm.*	*mgm.*	
Experiment 3, sand—41 days:							
0 8 per cent CO_2	8	12 6	10 5	254 ±23	885 ±85	22.33 ±2.15	2 53 ±0.03
0 4 per cent CO_2	8	14 5	9.8	264 ±25	740 ±92	19 44 ±2.24	2 65 ±0 08
0 2 per cent CO_2	10	13 6	7 4	195 ±13	585 ±69	16.90 ±1.71	2.89 ±0 07
0.1 per cent CO_2	10	13 3	8 3	200 ±12	520 ±52	15 35 ±1.41	2.98 ±0.04
Air	9	9 4	7 2	88 ± 7	247 ±35	7 50 ±0 85	3 04 ±0 07
Cotton plugs	4	8 5	6 5	66 ± 5	132 ±17	4 52 ±0.55	3.44 ±0.08
Open	3	10.7	7 7	164 ±20	690 ±66	18 53 ±1.33	2 69 ±0.21
Experiment 4, agar—54 days:							
0.8 per cent CO_2	10	15 4	9 1	254 ±13	1,192 ±61	27 3 ±1.2	2.28 ±0.05
0.4 per cent CO_2	8	12 5	9 5	209 ±11	959 ±37	22 6 ±1.2	2.35 ±0 07
0.2 per cent CO_2	8	11 3	9 5	332 ±35	890 ±47	20 2 ±1 4	2 24 ±0 05
0.1 per cent CO_2	8	11 7	10 3	281 ±20	690 ±27	17 7 ±0 09	2 56 ±0.06
Air	9	9 5	10.6	136 ± 9	271 ±10	9 47 ±0 22	3.48 ±0 06
Cotton plugs	9	7 5	9.6	52 ± 6	129 ± 9	4 97 ±0 35	3 06 ±0 26

to be significant. Although the difference in the means of two adjacent series, i.e., 0.1 per cent and 0.2 per cent CO_2, or 0.4 per cent and 0.8 per cent are not always significant, it is apparent that the greater the pCO_2 in the atmosphere supplied the plants, the greater is the dry weight and the nitrogen fixed. Because of the large variation in the individual responses, this would not be apparent if only two adjacent series were compared, but when the experiment is considered as a whole there can be little doubt but that each increase in the pCO_2 resulted in an increase in the dry weight and in the nitrogen fixed. Of particular interest are the data on the percentages of nitrogen in the plants; they show that not until the CO_2 concentration reached 0.4 per cent was there

TABLE 3

Summary of the probabilities that the differences observed in the means arose from chance

Experiments 3 and 4

TREATMENT	0.1 PER CENT CO_2				0.2 PER CENT CO_2				0.4 PER CENT CO_2			
	Number nodules	Dry weight	Total N	Per cent N	Number nodules	Dry weight	Total N	Per cent N	Number nodules	Dry weight	Total N	Per cent N
0.1 per cent CO_2:												
Experiment 3					0 75	0 25	0 45	0 20	0 02	0 04	0.10	<0.01
Experiment 4					0 20	<0 01	0 15	<0 01	<0 01	<0.01	<0.01	<0.01
0.2 per cent CO_2:												
Experiment 3	0 75	0.25	0 45	0.20					0 02	0 20	0.30	0.03
Experiment 4	0 20	<0.01	0 15	<0 01					<0 01	0 30	0.35	0.15
0.8 per cent CO_2:												
Experiment 3	0 03	<0 01	0.01	<0 01	0 03	0 01	0 07	<0 01	0 75	0 25	0 30	0.20
Experiment 4	0 04	<0 01	<0 01	<0 01	0 01	<0 01	<0 01	0 50	0 20	<0 01	<0.01	0 30
Air:												
Experiment 3	<0 01	<0 01	<0 01	0 40	<0 01	<0 01	<0 01	0.12	<0.01	<0 01	<0.01	<0.01
Experiment 4	<0 01	<0 01	<0 01	<0 01	<0 01	<0 01	<0 01	<0 01	<0.01	<0.01	<0.01	<0.01

any significant change in the dry weight-nitrogen relation. This would indicate that, to a certain limit, the large increases in carbohydrate formation by the plants were accompanied by corresponding increases in the nitrogen fixed. Beyond this limit the differential between photosynthetic activities and respiration proceeded at a more rapid rate than did the nitrogen fixation process.

The plants grown in bottles closed with a cotton plug were lower in dry weight and nitrogen than were any of the rest; of especial interest is the fact that the percentage of nitrogen in these plants is much higher than it is in plants of other series, indicating that because of the low pCO_2, carbon assimilation is the limiting process in the development of plants grown in cotton-plugged containers. The data on the plants grown in the open bottles support the observation already made: if additional CO_2 is supplied to plants grown in a closed container, they are able to overcome the handicap imposed by their unnatural habitat. However, this does not mean that the cause of the poorer growth noted in closed systems is lack of CO_2, but that by stimulation of the carbon assimilation with increased CO_2, the handicaps in growth imposed on the plant by the closed container can be overcome.

Experiment 4

Experiment 4 was a repetition of experiment 3 except that the plants were grown on Crone's agar instead of sand. The cultures were planted with red clover and inoculated with *Rh. trifolii* 209 on September 13, 1931. They were allowed to grow for 17 days before being connected to the manifolds; at this time the plants had their first leaves and some nodules were formed. Soon after the various gas mixtures were started, the plants receiving additional CO_2 begin to show signs of carbohydrate excess; the stems became red, the leaves turned yellow, and it was evident that the plants were suffering for lack of nitrogen. This period lasted for about 10–15 days, then the plants suddenly recovered and in 2 weeks had filled completely the 64-ounce bottles. It was apparent that the additional CO_2 was of no benefit and was possibly detrimental to the plants unless the nodules had developed to a point at which they could supply sufficient nitrogen for the needs of the growing plant. It is significant that this period of nitrogen starvation is not nearly so apparent in plants grown in sand and supplied with increased CO_2, which probably means that there is some factor which limits the nitrogen fixation process in plants grown in agar. The plants were harvested when 54 days old; during the last 2 weeks of their growth, artificial lights were used to supplement the sunlight.

The plants receiving increased CO_2 were stocky and thrifty, dark green, pubescent, and with some reddening of the stems. The stems were heavy, the leaves very large, and the roots were short and thick. They could hardly be distinguished from plants grown in open pots. The plants receiving the higher concetrations of CO_2 were somewhat larger and showed more carbohydrate excess than did the others. The nodules on the 0.4 per cent and 0.8 per cent CO_2 series were very large and placed on both the tap and the lateral roots. There

were more nodules on the 0.1 per cent and the 0.2 per cent CO_2 series, but many of these were the small, round, scattered type. The plants supplied with air were not nearly so large as the ones receiving additional CO_2, but they were much better than those grown in bottles closed with cotton plugs. The nodules on the plants supplied with air were small to medium in size and were found on both the tap and the lateral roots; in the cotton plugged series, the nodules were few in number and located near the crown on the taproot.

The results of the analyses are given in table 2, experiment 4. The means of the variables measured were tested for significance by the analysis of variance; as in experiment 3, the populations were found to be non-homogeneous, indicating that the treatments produced a significant effect. The observed differences in the means were further tested by the modified "Student" method; the results of this test are summarized in table 3.

The results are very similar to those of the sand series; with increasing CO_2, there is an increase in size, dry weight, and total nitrogen in the plants. Although the difference between the means in adjacent series is not always significant there is a definite trend toward higher values of the variables measured as the pCO_2 supplied is increased. In general the results of this experiment are little different from those of experiment 3 but there is one outstanding difference—the percentage of nitrogen in the plants. Although both the dry weight and nitrogen fixed by the plants grown under a given pCO_2 is somewhat higher in the agar series than is the corresponding value in the sand series, the percentage nitrogen in the plants is, in general, somewhat lower. Moreover, in the case of clover grown on sand, there was no decrease noted in the percentage of nitrogen in the plants until the 0.4 per cent series was reached, but with clover grown in agar, an increase in the CO_2 content from 0.03 to 0.1 per cent resulted in a significant decrease in the percentage of nitrogen. An increase from 0.1 to 0.2 per cent in the CO_2 content of the atmosphere caused a slight decrease in the value of this figure, but further increases in the CO_2 content were without effect on the dry weight-nitrogen relation. As observed in experiment 3, the percentage of nitrogen in the plants with a carbon dioxide deficient atmosphere (cotton plugs) is higher than in any other series.

Experiment 5

The results of experiments 1 to 4 have established the importance of the photosynthetic process to nitrogen fixation by clover when grown in a closed system. The use of closed bottles for plant containers is convenient since this simplifies the control of the gas mixtures furnished the plants, but it is believed that the major findings of experiments 1–4 should be checked for the case of plants growing in the open. Accordingly, an experiment was made in which additional CO_2 was supplied clover plants which were grown in the 64-ounce bottles open to the air. The plants were placed in the greenhouse November 11, 1931, and aeration with an air-CO_2 mixture and with air alone started immediately; the rate of aeration was 100 cc. per minute per bottle. Artificial illumination was used to supplement sunlight throughout this experi-

ment. The average CO_2 content of the air inside the bottles supplied with this gas was 0.5 per cent, whereas that of the air alone was 0.033 per cent. The age of the plants at time of harvest was 48 days.

The plants in the series which received the CO_2 plus air mixture were very large, stocky, and showed some carbohydrate excess; the leaves were very large and dark green. The plants protruded 3 to 4 inches from the bottles and several were blooming. All were well nodulated, but the nodules were somewhat scattered throughout the plant system. The plants which were aerated with air alone were not quite so stocky as the series receiving CO_2 but were inclined to be tall and green. There were not so many nodules on the roots and only one plant was blooming. The plants grown in open bottles without aeration were very similar to those aerated with 0.03 per cent CO_2 (air).

TABLE 4

The effect of added CO_2 on clover plants grown in open bottles

Experiments 5 and 6

TREATMENT	NUMBER OF SAMPLES	LENGTH OF TOPS	LENGTH OF ROOTS	NUMBER OF NODULES	DRY WEIGHT	NITROGEN	
						Total	Per cent
		cm.	*cm.*		*mgm.*	*mgm.*	
Experiment 5—48 days:							
Air plus CO_2	7				1,255 ±87	30 4 ±1 7	2 42 ±0 14
Air	7				765 ±45	20 7 ±1 0	2 72 ±0 09
Not aerated	5				587 ±38	15 9 ±0 9	2 71 ±0 04
Experiment 6—45 days:							
Air plus CO_2	7	19 7	12 0	179 ±18	1,384 ±58	34 5 ±1 7	2 49 ±0 06
Air plus CO_2 and nitrate	5	16 2	12 6		1,136 ±46	23 7 ±1 5	2 08 ±0 19
Air	7	15 7	9 4	104 ± 7	762 ±35	21 8 ±0 9	2 87 ±0 04
Air plus nitrate	5	15 8	11 8		780 ±21	22 7 ±0 9	2 92 ±0 13
Not aerated	6	16 0	10 0	112 ±10	747 ±32	22 7 ±1 1	3 05 ±0.12

The data, which are presented in table 4, experiment 5, confirm the results obtained in the previous experiments. The dry weights of the plants given additional CO_2 were increased about 65 per cent and the nitrogen fixed was increased about 50 per cent. As a result, the percentage of nitrogen in the plants receiving air plus CO_2 was somewhat lower than in those aerated with air, or in those merely open to the air.

Experiment 6

In this experiment, which was a repetition of experiment 5, some of the plants were supplied with combined nitrogen in order to compare their growth with plants fixing nitrogen. The plants were set up on December 13, 1931. Some were inoculated with *Rh. trifolii* 209 and others were supplied with combined nitrogen as NH_4NO_3 periodically during the growth period. An attempt was made to add the NH_4NO_3 at the start at a rate that would allow the inoculated plants to keep pace with those supplied with combined nitrogen.

After nitrogen fixation was well under way in the inoculated plants, the NH_4NO_3 was added in excess to the *nitrate series* in order to determine whether the nitrogen fixation process was limiting the growth of the inoculated plants. The addition of the NH_3NO_3 was as follows: 7 days, 2 mgm.; 15 days, 5 mgm.; 21 days, 5 mgm.; 28 days, 5 mgm.; 35 days, 10 mgm.; 38 days, 5 mgm.; 40 days, 2 mgm.; 42 days, 5 mgm.; 44 days, 3 mgm. A total of 45 mgm. of combined nitrogen per bottle was added to the nitrate series but algae grew in all of the cultures so that the actual amount available to the plants at any time was lower than the additions indicate. The plant cultures were not aerated until 20 days after planting in order to allow nitrogen fixation to start before the increased pCO_2 was supplied the plants. After 25 days of aeration, the plants were harvested.

The inoculated plants which received CO_2 were large, dark green, and showed some signs of carbohydrate excess. The nodules were predominantly large on the taproot with numerous medium and small nodules scattered on the lateral roots. The plants that were supplied with nitrate and additional CO_2 were very similar in appearance to the inoculated plants. The inoculated plants aerated with 0.03 per cent CO_2 (air) and those which were merely open to the air were somewhat smaller than the plants in the CO_2 series. They were dark green and showed no indications of carbohydrate excess. The nodules were large and located almost exclusively on the taproot. The nitrate controls of this series also had smaller plants than those supplied with additional CO_2.

The analyses are given in table 4, experiment 6. In general they confirm the results of experiment 5, i.e., an increased pCO_2 in the atmosphere supplied plants grown in the open results in a large increase of dry weight and a somewhat smaller increase in the quantity of nitrogen fixed. The nitrate controls of the plants receiving CO_2 did not assimilate as much nitrogen as was fixed by the inoculated plants, but this might have been due to the competition for the added nitrate by the algae growing in these cultures. A comparison of the effect of increased CO_2 on plants fixing nitrogen and those supplied with combined nitrogen is being made under more controlled conditions.

Experiment 7

In all of the previous experiments, red clover was used as the experimental plant. One experiment was made in which alfalfa was grown on sand in an atmosphere containing 0.2 per cent CO_2 and compared with control plants in bottles closed with a cotton plug (less than 0.03 per cent CO_2). The results were practically identical with those obtained with clover plants; the dry weight of the plants supplied with CO_2 was almost 3 times that of the controls, whereas the increase in nitrogen fixed was about 2.5 fold.

DISCUSSION

General

The results of seven experiments in which over 200 plant cultures were used demonstrated conclusively that nitrogen fixation by red clover in association

with its specific organism, *Rh. trifolii*, could be markedly increased by growing the plants in an atmosphere containing a higher partial pressure of CO_2 than that of air. Plants grown on sand plus modified Crone's solution in an atmosphere deficient in CO_2, e.g., in a cotton plugged bottle, have a high percentage of nitrogen indicating that the carbon assimilation and not nitrogen fixation is the process limiting the growth of the plant. Under these conditions the percentage of nitrogen in a plant is between 3.0 and 3.5 per cent; if plants are grown in a closed system and supplied with normal air (0.03 per cent CO_2) the percentage of nitrogen in the plant decreases to slightly less than 3 per cent, but the dry weight and total nitrogen are increased. Further increases in the pCO_2 in the atmospheres furnished the plant results in 100–200 per cent increases in both dry matter and nitrogen fixed with no change in the percentage nitrogen in the plant until atmospheres containing between 0.2 and 0.4 per cent CO_2 are used. As the CO_2 content is increased beyond these figures, there is a proportionately greater assimilation of carbon than of nitrogen so that while the absolute value of the nitrogen fixed increases, the percentage of nitrogen in the plant decreases. This is accompanied by typical signs of carbohydrate excess in the plants, i.e., reddening of the stems and leaves. Thus by increasing the pCO_2 in the atmosphere supplied to inoculated clover plants grown in a closed system the metabolism of the plant can be changed from a type in which carbon assimilation is the limiting factor in development, through a stage in which carbon assimilation and nitrogen fixation are in equilibrium, to a final stage in which nitrogen fixation becomes the factor limiting the growth.

With plants grown in agar, the situation is much the same except that any increase in the pCO_2 results in a decrease in the percentage of nitrogen in the plant, although the *absolute* quantity of nitrogen fixed is increased. That is, if the carbon dioxide content of the air supplied to plants grown in agar is only slightly increased, carbon assimilation (differential between photosynthesis and respiration) takes place at a more rapid rate than does nitrogen fixation. With plants grown in sand, this effect is not observed until the CO_2 content of the atmosphere reaches about 0.4 per cent. The cause of this difference is not readily apparent but it is likely concerned with the lowered gas exchange of the roots of plants in agar. If this lowered gas exchange causes a deficiency in the supply of oxygen to the nodules, less respiration will take place with consequent increase in the differential between photosynthesis and respiration; also, the deficiency in oxygen may be a limiting factor in the fixation process as suggested by Thornton (22). It is likewise possible that poor gas exchange affects the nitrogen fixation process of plants in agar by a decrease in the pN_2 in the nodule; or by an accumulation of toxic products, including CO_2, in the nodules caused by the accelerated respiration of the bacteria in the presence of an increased supply of carbohydrate.

A second effect of increasing the pCO_2 available to clover plants fixing nitrogen is the changes induced in the number, size, and distribution of the nodules.

Plants grown in a CO_2-enriched atmosphere show 2 to 3 times as many nodules as the controls in air, and the size is greatly increased; in some cases the increased size appears almost pathological. However, more striking than either the increase in the number or size of the nodules is the change in distribution. Ordinarily the nodules are clustered around the crown on the taproot, but as the pCO_2 in the atmosphere rises there is a tendency to invade the lateral roots, so that plants grown in relatively high concentration of CO_2 (0.5 per cent) have a root system in which the nodule distribution is very similar to that of plants inoculated with poor strains of the organisms. This development of nodules on the secondary roots suggests that possibly invasion of these roots takes place in all plants, but because of limited carbohydrate supply, the organisms in the lateral roots are kept from developing and no nodules appear. A poor strain would then be an organism in which parasitism had been accentuated to a degree that it could compete with the plant for its carbohydrate even when this is present at a low level in the plant sap. On the other hand, the invasion of the lateral roots on plants high in carbohydrates may mean only that the chemotaxis of the invasion of roots is related to the carbohydrate metabolism of the plant.

Theoretical

The results of the experiments described in the text throw some light on the mechanism of the fixation process by the association of the plant and bacteria. Because of the inability to demonstrate fixation by the bacteria apart from the host plant, there has been engendered some doubt as to whether or not the bacteria are directly concerned in the fixation process. The picture presented by plants receiving additional CO_2 suggests that the bacteria are the actual agents concerned in the fixation. The period of lag during which the plant nearly succumbs because of the lack of nitrogen is suggestive of the development of bacterial tissues in the nodule independent of the plant activities. If the plant were the primary agent concerned in the fixation, it does not appear likely that its carbon assimilation and nitrogen fixation functions would be so completely independent that there could occur such a displacement of the carbon-nitrogen equilibrium as is observed. On the other hand, if the bacteria are the causal agents in the fixation but are dependent on the plant for a source of energy, the nature of the stimulative effects of increased photosynthesis by the plant becomes apparent. Another observation that tends to link the bacteria with the actual fixation process is that made on the size and number of the nodules. In those plants which developed a large number of nodules when supplied with additional CO_2, the size of the nodules formed was not greatly increased; other plants responded to CO_2 treatment by an increase in the size of the nodules rather than an increase in the number. Thus it appears that the need for additional nitrogen is met by either development of new centers of nitrogen fixation, or by increased activity on the part of the centers already present. This evidence of a correlation in the development

of the bacteria in the plant with the quantity of nitrogen fixed, indicates that the function of the bacteria in the process is something more than a mere stimulation of the plant. Examination of the nodules on plants supplied with increased CO_2 shows that they are filled with bacteriods much larger than those found in nodules of plants supplied with air alone. It is planned to make cytological studies of these nodules in an effort to obtain more information in regard to the effect of the increased supply of carbohydrate on the bacteriods and nodular tissue.

There is still to be answered the question in regard to the specific nature of the observed stimulation of the nitrogen fixation process by an increase in the carbon assimilation of the plant. The obvious answer is that the increase in the rate of photosynthesis gives rise to a higher level of carbohydrate in the sap of the plant, which furnishes the bacteria with the energy necessary for an increase in the fixation of nitrogen. Although this sounds plausible, it is possible that the function of the plant is to furnish not merely a source of energy but specific compounds, intermediate in its carbohydrate metabolism, which are required by the bacteria by reason of the mechanism chosen for the fixation process. For example, if the first compound formed in the fixation is the result of an equilibrium reaction, the fixation process is conditioned by the removal of this compound. This might be done by combination with some specific intermediate of the carbon assimilation process, and in such a case the concentration of this intermediate in the plant would be the factor limiting the fixation of nitrogen by the bacteria.

Practical application

The discussion has dealt exclusively with the theoretical aspects of the results of the experiments reported, but equally important are the practical applications to agricultural science. In view of the marked stimulation of nitrogen fixation by clover and alfalfa plants supplied with additional CO_2, it might prove decidedly advantageous for the practical agriculturist to adopt methods of soil culture which will cause an increase in the pCO_2 in fields cropped to these leguminous plants. Although the use of commercial sources of CO_2 is probably impractical, the application of organic manures and the necessary cultivation for maximum activity of soil microorganisms would very likely prove a most economic investment in carbon dioxide production. Field experiments in which the possibility of forcing an increased synthesis of nitrogen by leguminous plants through application of various types of CO_2 manuring might prove of decided value to agricultural science.

A second possible application of these data is to those experiments in which nodule production is used as a criterion of the efficiency of a culture of rhizobia, e.g., the testing of commercial cultures. Ordinarily, this testing is done on a sterilized sand substrate in which carbon dioxide production is at a minimum, whereas the cultures are intended to be used in soils producing appreciable quantities of CO_2. In view of the results on the influence of pCO_2 on the num-

ber of nodules, it might be profitable to compare cultures under conditions such that the carbohydrate metabolism of the plant can not be the limiting factor in the production of nodules.

SUMMARY

A review of the literature which deals with the effects of supplying additional CO_2 to plants, indicate that leguminous plants are especially responsive to an increase in the pCO_2 of the atmosphere in which they are growing. The relation of this observation to the fixation of atmospheric nitrogen by the associated growth of these plants with bacteria has been investigated.

A method has been developed for the estimation of sand adhering to the roots of plants which are to be used for the determination of total nitrogen. It consists of digesting the plant material by the usual Kjeldahl procedure, diluting with 250 cc. distilled water, then comparing with suitable "sand standards." These standards are prepared from sand-free plant material to which is added a known weight of sand. The maximum error in the determination is 10 to 15 per cent; with this accuracy, the error in the estimation of the dry weight of plants is less than 2 per cent.

Inoculated clover plants were grown on agar and sand in a closed system and provided with atmospheres which contained 0.03, 0.1, 0.2, 0.4 and 0.8 per cent CO_2. The effect of the different pCO_2 on the general growth and nitrogen fixation was determined by suitable measurements. In general as the pCO_2 in the atmosphere is augmented, the leaf area, length of tops, stockiness of plant, excess of carbohydrate, and number of nodules formed are increased. Raising the CO_2 content of the atmosphere from 0.03 (air) to 0.1 per cent results in a pronounced increase in these characteristics of plant growth, but further increments in the pCO_2 do not induce correspondingly greater development.

Clover plants, grown in agar and which were supplied with atmospheres containing 0.1 per cent or more CO_2, show an increase in both the dry weight and the quantity of nitrogen fixed, when compared with the controls supplied with air. However, the gain in nitrogen is relatively less than that in dry weight, and this difference causes the plants receiving additional CO_2 to have a lower *percentage of nitrogen* than the controls. With plants grown in sand, the lag in nitrogen fixation as compared with carbon assimilation does not occur until the CO_2 content of the atmosphere is raised to about 0.4 per cent. This difference between plants in agar and in sand, when furnished with small increments of CO_2, is believed to be related to the lower rate of gas exchange of the roots of plants in agar.

In addition to an increase in the number of nodules, addition of CO_2 results in a change in the size and distribution. Plants supplied with the higher partial pressures of CO_2 often exhibit nodules which are enormous in size, especially those located on the taproot. Invasion of the lateral roots also occurs so that the distribution of nodules on the root system becomes definitely similar to that associated with infection by poor strains of the organism.

The major findings, obtained with clover plants in a closed system, have been verified for clover planted in bottles open to the air and for alfalfa grown in the closed system.

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BOOK REVIEWS

Root Nodule Bacteria and Leguminous Plants. By EDWIN BROUN FRED, IRA LAWRENCE BALDWIN, AND ELIZABETH MCCOY. University of Wisconsin Studies Number 52, Science No. 5. The University of Wisconsin, Madison, Wisconsin, 1932. Pp. XXII + 343, pl. 46, charts 4; paper cover. Price $3.

This is a comprehensive monographic treatise covering the numerous phases of research concerned with legume bacteria and legumes. Chapter headings will suggest the nature of organization of the material: 1. The history of leguminosae in agriculture (ancient use of legumes and discovery of bacterial association). 2. General description of the leguminosae, their distribution and importance (includes composition). 3. The occurrence of root nodules (discovery, importance, characteristics). 4. The isolation and study of the root nodule bacteria (methods, occurrence, contaminants in the nodule). 5. The morphology and life cycle of the root nodule bacteria. 6. Cultural and biochemical characteristics of the root nodule bacteria (nutrition, nitrogen-fixation, action of bacteriophage, composition of the bacteria). 7. Some factors which influence the growth and longevity of the nodule bacteria (environmental conditions as oxidation-reduction, aeration, temperature, desiccation, sunlight, reaction, organic and inorganic substances). 8. Species relationships (classification and differentiation of species, cross inoculation groups, nomenclature). 9. The formation of nodules, their histology and cytology. 10. Relationship between leguminous plants and bacteria (strain variation, physiological efficiency, parasitic strains of the bacteria, mechanism of nitrogen fixation). 11. Factors that influence nodule production (environmental conditions and nodule formation—aeration, moisture, light, temperature, reaction, inorganic salts, nitrogenous substances, organic materials). 12. Economic importance of leguminous crops (gains in nitrogen by growth of legumes, association of legumes and non-legumes). 13. Natural and artificial inoculation (methods of inoculation—soil and pure culture methods). There follows an appendix in which is discussed the preparation, distribution, and testing of artificial cultures of root nodule bacteria. There are a complete list of references and an index.

In soil microbiology there is scarcely a chapter which is more impressive than that dealing with the nodule bacteria. This is partly accounted for by the outstanding achievements of an illustrious body of investigators who have worked in the field and partly by the immense practical benefits which have followed the application of principles established in these researches.

The voluminous literature on legume bacteria bears witness to the intense interest in the subject.

Fifty years of probing have brought forth reports in technical journals, bulletins and whole volumes in English, German, French, Italian, Dutch and the Scandinavian languages, to mention only the most common sources of material upon the Leguminosae. So much study in a comparatively narrow, though vital and perplexing field have often quite naturally "—resulted in unconsciously going over the ground already known and recorded by different investigators."

A project which aims to gather together the published reports of such a subject, to organize it, and to present the details comprehensively and impartially is undoubtedly meritorious. It is doubtful whether any persons are better qualified to undertake this work than the authors, long experience with numerous ramifications of the subject enabling them to speak with authority and to judge debatable points keenly.

Even a casual examination indicates that the volume is excellently arranged; more detailed examination reveals the care and thoroughness with which the authors present the facts. They have preserved a fine balance in the material, eliminating any over emphasis of their own extensive researches. The plates are excellent.

The far-reaching influences of the monograph are difficult to estimate. It seems inevitable that it should accomplish the authors' desire to avoid repetition and focus attention on fruitful unexplored branches of research. It likewise appears to have value no less important in correcting false or uncertain opinions which appear in discussions of legumes and legume bacteria.

It is hoped that others will be inspired to do as much for various branches of soil microbiology.

ROBERT L. STARKEY.

Fixed Nitrogen. Edited by HARRY A. CURTIS. American Chemical Society Monograph Series. The Chemical Catalog Company, Inc., New York, 1932. Pp. 517, figs. 82.

The book is one of a series of scientific and technologic monographs published under the auspices of the American Chemical Society. In the General Introduction, the board of editors of the American Chemical Society say: "Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line." They say further: "The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended."

In outlining the scope of the book, the editor states; "The present monograph presents first a brief survey of the general situation with regard to the distribution of the earth's total nitrogen and of the methods by which the free nitrogen of the atmosphere is made available in combined or 'fixed' form."

The book contains 16 chapters, pp. 11–490; a bibliography, pp. 491–506, and author references, pp. 507–513. There are 4 chapters by Curtis, viz: I. General Survey of the Sources and Utilization of Fixed Nitrogen: III. The Chilean Nitrate Industry: IV. Fixed Nitrogen Recovered in Coal Carbonization; and V. A History of Nitrogen Fixation Processes. Chapter II. Nitrogen Fixation by Living Organisms, was contributed by F. E. Allison; Chapter VI. Physical Methods of Studying Chemically Active States of Gases and of Investigating Catalyst Surfaces, by C. H. Kunsman; Chapter VII. The Arc Method of Nitrogen Fixation, by Norman W. Krase; and Chapter VIII. Synthetic Ammonia, by P. H. Emmett. Chapter IX. Some Physical Properties of Hydrogen, Nitrogen, Methane, Ammonia, Carbon Monoxide and Carbon Dioxide and Their Mixtures, is by Richard Wiebe; Charter X. High Pressure Equipment and Technic, by J. R. Dilley and W. L. Edwards; and Chapter XII. The Alkali Cyanide Method of Nitrogen Fixation, by E. W. Guernsey. Three chapters were contributed by H. J. Krase, viz: XI. The Cyanamide Method of Nitrogen Fixation, XII. Synthesis of Urea, and XIV. Oxidation of Ammonia. Chapter XV. Synthetic Nitrogenous Fertilizers, was written by William H. Ross and Albert R. Merz, and Chapter XVI. Nitrogen Statistics by, P. E. Howard.

The contributors have furnished us with a long-range view of the world nitrogen resources. They have also supplied us with a historical record on the development of nitrogen-fixation processes, aside from an adequate discussion of the nature of the processes themselves. The work is a valuable addition to present-day reference material on the subject of fixed nitrogen.

Methoden für die Untersuchung des Bodens. Part I. Edited by O. LEMMERMANN. Verlag Chemie, Berlin, 1932. Pp. 90, ill. 2.

As is indicated by the title, the volume is devoted to a discussion of methods for the study of soils. It was prepared in behalf of the German agricultural experiment stations and the German Society of Soil Science. The five divisions of the book deal, respectively, with: A. the sampling of the soils, B. the physical examination of mineral and humus soils, C. the examination of mineral soils, D. the examination of peat soils, and E. examination of forest soils. The section devoted to sampling, outlines methods that would best serve the purpose of those interested in soil mapping and classification as well as in the study of physical, chemical, plant physiological, and microbiological attributes of soils. Methods are also described for the taking of samples of peat soils and of samples of soil profiles.

The second section concerns the mechanical analysis of soils and the determination of specific gravity, water-holding capacity, heat and wetting, and hygroscopicity. Section 3 is divided into three parts, dealing respectively with the chemical examination of soils, the determination of the fertilizer requirements, and microbiological studies. Methods of determining total and so-called available plant-food are given and likewise older and more recent

methods which concern the calcium status of soils and the determination of so-called available nutrients by plant physiological and chemical methods. Among the methods described are those of Mitscherlich, Neubauer, Schneider, König Hasenbäumer, Lemmermann, Fresenius, and 'Sigmond. There is a reasonably complete description of methods dealing with the determination of microbiological properties of soils. The same may be said also of section D, where methods for the study of peat and muck soils are described. No methods are specifically given for the examination of forest soils. The editor suggests that until such methods are more fully established those given for soils in general be employed.

The book, which is really a laboratory manual, will be found useful by students of soils, who will no doubt appreciate both the text and the reference to the appropriate literature.

Land Drainage. By W. L. POWERS AND T. A. H. TEETER. Second Edition, Revised and Enlarged. John Wiley & Sons, Inc., New York and London, 1932. Pp. x + 353, figs. 169.

The book is divided into four parts, dealing respectively with field drainage, district drainage, special drainage problems, and drainage surveying. There is also an appendix, which deals with farm drainage laboratory exercises, tables for use in Kutter's Formula, and U. S. Census of Drainage—1930.

In the Foreword of the first edition—which appeared in 1931, the author, W. L. Powers, pointed out that the "volume is intended, first, as a text book for students of general agriculture or agricultural engineering; second, as a reference book for practical farmers; and third, as an aid to owners of wet, overflowed, marsh, swamp or alkaline land who desire to improve their holdings." In the Introduction to the second edition, the senior author notes that "During the decade which has passed since the first edition was prepared, a number of notable developments have occurred in the drainage field. River and flood control have required attention of the national congress; soil erosion and terracing has become a problem of national importance; war salvage explosives have been extensively used in drainage and clearing; drainage in irrigated sections by means of pumping from wells has found wide application; progress has been made in developing pumping and excavating machinery, in building ditches with the aid of a scarifier in connection with running water, and in the use of vertical drainage."

Aside from the technic of field drainage dealt with in Part 2, there is a very helpful discussion of drainage districts and drainage laws, of flood control, and of allied topics. Among the special drainage problems, the authors discuss the drainage of tidal and overflowed marsh lands and of irrigated lands, and likewise drainage by means of pumping wells, prevention and control of erosion, with special reference to terracing. Part 4 deals with drainage surveying and practices.

The book will be found useful as a text on land drainage as well as a reference book for teachers and land owners.

Der Einfluss der Handelsdünger auf das Pflanzenwachstum und auf verschiedene Eigenschaften Kalkarmer Mineralböden. By LUDWIG SCHMITT with an introduction by HUBERT RÖSSLER. Verlagsgesellschaft für Ackerbau M. B. H., Berlin, 1932. Pp. 186, ill. 12, graphs 20.

The rapid expansion in the manufacture of synthetic ammonia has resulted in an increased consumption of ammonia salts. This increase in consumption has been particularly noteworthy in Germany. As a result of the continued use of ammonium sulfate and of other ammonia salts, soil acidity has become so pronounced in some sections of Germany as to have led to the studies recorded in this book. The volume is divided into six sections, dealing respectively with an introductory statement; the physiological reaction of potassium salts; the physiological reaction of phosphorus fertilizers; the physiological reaction of nitrogen fertilizers; the action of potash fertilizers as indicated by long-continued field experiments at the Darmstadt Station; and the determination of the lime requirements of soils. There is also a concluding paragraph by the author and a reference list containing 86 items.

Fertilizers and Crop Production. By L. L. VAN SLYKE. Orange Judd Publishing Company, Inc., New York. 1932. Pp. xiv + 493, frontispiece, ill. 70, tables 63, appendix.

The present volume represents a revision of "Fertilizers and Crops" by the same author, published in 1921. It is stated by the author in his preface: "In the preparation of this book, it has been the author's aim to keep in mind not only the requirements of students in agricultural colleges and high schools but as well the needs of student farmers who, in increasing numbers, are directly and vitally concerned in the profitable production of crops. The book can be used to advantage in the educational work promoted by granges and other farmers' organizations."

The five parts of the book deal respectively with factors of soil fertility; functions and physical properties of soils; sources and composition of materials used as fertilizers; factors that guide the selection of fertilizer materials; and practical use of fertilizers in growing individual crops. Like its predecessor, this book is extremely valuable in supplying the teacher, research worker, and extension specialist with extensive and authoritative information on the composition of soils, fertilizers, and plants. Beyond that, there is a sound and wholesome philosophy which finds expression. This may perhaps be best illustrated by the following statement of the author. "Generally speaking, the fundamental purpose of this book is to study the conditions under which plant-foods, whether in the form of soil compounds, of farm-produced materials, or of commercial fertilizers, can be conserved and at the same time utilized with the greatest efficiency and economy in the production of crops."

Soils. Their Origin, Constitution, and Classification. By GILBERT WOODING ROBINSON. Thomas Murby & Co., England, and D. Van Nostrand Co., New York. 1932. Pp. xv + 390, frontispiece, figs. 12, tables 38.

The author, who is professor of agricultural chemistry in the University College of North Wales, has been for many years a careful student of the genesis, formation, classification, and mapping of soils. As the author himself notes: "I have written primarily for those who are interested in the soil as an object of study in itself, and secondarily for those whose interest lies in its economic or geographical significance; but it may be hoped that the book will prove of value to botanists because of the importance of the soil in ecology, and to geologists because of the part played by the soil in the weathering cycle."

The main body of the book consists of 19 chapters and an appendix devoted to methods of analysis. The designations of its several chapters are: Introductory; General View of the Constitution of the Soil; The Pedogenic Processes; The Clay Complex; Base Exchange and Other Reactions of the Colloidal Complex; Soil Organic Matter; General Physical Properties of Soils; Water Relationships of Soils; Soils of the Podsolic Group; Tshernosems and their Related Groups; Ground-water Soils, Including Peats; Saline, Alkaline and Soloti Soils; Soils of the Humid Tropics and Subtropics; Soils Associated with Calcareous Parent Materials; The Classification of Soils; The Geography of Soils; Soil Surveys; Soil Analysis; Soils, Plant Growth and Agriculture.

The author is to be commended for his effort to establish a wider basis of soil classification and mapping, and to create a clearer conception of soil factors in relation to world agriculture. This work will be welcomed by the teacher and students of soils and should find a place in every modern library.

pH and its Practical Applications. By FRANK L. LAMOTTE, WILLIAM R. KENNY, AND ALLEN B. REED. The Williams & Wilkins Co., Baltimore, Md. 1932. Pp. vii + 262, charts 18, figs. 15, tables 27.

"In this work," say the authors, "we have endeavored to set forth the fundamental principles of hydrogen ion control in a manner that would prove helpful to the operating chemist. No attempt has been made to present a discourse on the scientific phases of hydrogen ion measurements, since this has already been covered by eminent authors. We have endeavored to bear in mind the desires of the practical technologist, who is aware of the value of pH control, but who merely wishes a working knowledge of its application, rather than a thorough treatise of the subject as a pure science."

Section A of the book is entitled "Mechanism of Hydrogen Ion Determinations" and contains chapters on introduction and preliminary discussion; hydrogen and hydroxyl ions; buffer action; determination of pH; sources of error. Section B deals with "Practical Application of Hydrogen Ion Determinations in Industry and Science," and contains chapters on municipal and industrial water supply; water corrosion problems; disposal of sewage and industrial waste; sugar industry; gelatin and glue; leather manufacture; textile industry; pulp and paper industry; food industries; cleaning processes; electrodeposition of metals; general industrial chemistry; bacteriology, pathology and titration processes; and soils.

The authors have rendered a distinct service to laboratory workers by bringing together in readily usable form information on hydorgen ion determinations and appropriate equipment for such determinations.

Der Kationen- und Wasserhaushalt des Mineralbodens. By P. VAGELER. Verlag von Julius Springer, Berlin, 1932. Pp. vi + 336, ill. 32, chart 1.

The principal portions of the book deal with the outlining of problems and objectives, the physical, chemical, and biochemical processes in soils; and methods of research and analysis. There are a comprehensive bibliography of 398 items and author and subject indexes. A large chart at the end of the book is entitled: "Übersichtstabelle wichtiger Bodentypen der Erde in Lösungs- und Komplexbau."

The author notes in the preface that soil science as an independent science is relatively new, and that it has not been sufficiently rounded out to reflect fully world-wide conditions and relations. He notes, further, that there still exist soil science teachings distinctly national in character. There are often conflicting views and teachings, and, despite the influence of the International Society of Soil Science, there are but few teachings and conclusions which find acceptance beyond a small group of specialists. It is gratifying to find that this book, together with some other recent treatises, has contributed in a substantial way toward giving us a broader outlook on the field of soil science.

The Geology and Water Resources of Northwestern Minnesota. By IRA S. ALLISON. The University of Minnesota Press, Minneapolis, 1932. Pp. xii + 245, tables 96, figs. 36.

The book deals primarily with the physiography of the area, general geology and yield of water, and chemical character of the waters. Beyond that, water supplies of the 26 counties are considered by the author.

The book is a distinct contribution to the body of knowledge on water supplies and their character. The significance of the information contained in the book is well indicated by the data given in the several tables. For instance, Table I is entitled "Average of All Dissolved Solids in Waters of Northwestern Minnesota by Counties"; Table II, "Relative Salinity of Waters from Different Sources in Northwestern Minnesota"; and Table III, "Average Composition of Waters from Streams and Lakes of Northwestern Minnesota." The following tables contain original data of a similar character. In view of the great importance of water supplies, their sources, composition, pollution, and treatment in our complex economic and social life, the author deserves recognition for the information which he has collected and systematized.

JACOB G. LIPMAN.

INFLUENCE OF SOIL HYDROGEN-ION CONCENTRATIONS ON INFECTION BY *HETERODERA RADICICOLA* (GREEFF) MÜLLER[1]

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Observational evidence would appear to indicate for the root knot nematode, *Heterodera radicicola*, a tolerance to a rather wide range of soil reactions. The senior writer's personal observations in Florida, for example, have shown root knot to occur both in the rather alkaline soils overlying coral in the vicinity of Miami and in the acid pine flats in the interior of the state. In pineapple fields in Hawaii, likewise, root knot is known to occur in fields that are distinctly acid as well as in those that are on the alkaline side of the neutral point. No exact information as to hydrogen-ion concentration relations appears to be available, however. In an effort to obtain facts as to the influence of measured ranges of soil pH on infection, certain experiments were conducted at this station and the results are reported herein.

LITERATURE REVIEW

The general literature on hydrogen-ion concentration is tremendous. One has only to refer to Clark's (6) book on the subject to realize the complete impracticability of mentioning even the more important contributions in such a paper as this one. There is, likewise, an extensive literature on the more restricted field of effect of soil hydrogen-ion concentration on the growth of plants. Two recent papers that have come to our attention may justify mention in this connection. Wiggin and Gourley (13) in their work on the reaction of greenhouse soils to the growth of plants first review very beiefly a number of papers on soil pH in relation to plant growth and then give the results of their own investigations on greenhouse

[1] Published with the approval of the Director as Technical Paper No. 55 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

[2] Nematologist and associate nematologist respectively. The studies herein reported were made possible only by the full cooperation of several others of the technical staff of this station whose assistance is hereby gratefully asknowledged. The first study, for example, was conducted with soils with controlled range of hydrogen-ion concentrations prepared by Dr. C. P. Sideris of the physiology department. The second study was materially assisted at the start in the matter of collection of field soils by Mr. F. A. E. Abel of the chemistry and soils department. The coöperation of field managers of various sugar and pineapple plantations was likewise indispensable in this. Soil pH determinations were made through the courtesy of Dr. F. E. Hance of the Hawaiian Sugar Planters' Association, Experiment Station and Dr. O. C. Magistad of this station, most of them by the latter. Routine examinations of plants as to root knot infection were made by Miss Juliette Oliveira and Miss Erna B. H. Gittel of the nematology Department.

ornamentals. Wessels (12) deals in a similar way with various vegetables grown on Long Island. Much work has been done on the influence of soil pH on various organisms. Dr. Chapman (5) refers to several papers on the subject. Arrhenius (1) shows by experimentation that soil pH plays a very important part in the occurrence of two species of earthworms in the soil. Their activity is apparently limited to a very narrow range in neutral or slightly acid soil. A number of investigations have been conducted on infection of various plants by the root parasitizing fungi. Without attempting an exhaustive literature review, one paper alone, by Burkholder (2) on dry root rot of bean caused by *Fusarium martii* var. *phaseoli* Burk. is cited. In this instance the fungus was tolerant to as wide a pH range as the host plant would endure and root rot occurred throughout the range (pH 4.04 to 8.43).

The literature on effect of soil pH on nematodes is more limited. Chandler (3) states in general that the population of soil nematodes (species not specified) is greatly increased in soil of high acidity (pH 5.2 to pH 6.0). Chandler (4) in his book on parasitology does not mention the possible influence of soil reaction on survival of nematode parasites in the soil. Definite investigations have been conducted on the influence of soil pH on the sugar beet nematode *Heterodera schachtii*. Peters (10) goes into great detail in an exposition of methods of determining soil hydrogen-ion concentrations and of the "pH" method of recording them. He then gives the results of measurements of soil pH in a series of nine plots and of an attempt to correlate these readings with *H. schachtii* cyst counts in the same areas. He does not actually work out the coefficient of correlation between the two variables, but shows in tabular form a sort of rank-order correlation. The hydrogen-ion concentrations determined in his test were from pH 6.0 to pH 6.7, a range of only 0.7 pH. He says, "From the table of averages it is clear that no very exact relationship obtains between the pH of the soil solutions and the cyst concentrations." However, he concludes that "—samples, from an experimental field at Kirton show an indubitable correlation between pH and cyst concentration. The cysts are very numerous in samples giving solutions of pH: 6.0; and few in samples giving solutions of pH: 6.7. Miscellaneous samples from South Lincolnshire in part confirm this correlation; but in a few cases a reverse correlation obtains." Again, "Were this advice followed (liming of sour soils) it is probable that the prevalence of *H. schachtii* would be greatly reduced—at least so far as concerns the strain found on potatoes."

Smith (11) likewise reports on *H. schachtii* in potatoes in another part of England and details an attempt to determine the presence or absence of a definite correlation between soil pH and cyst count. In one field of peat soil there appeared to be "a general trend indicating a negative correlation between pH and cyst count." The figures for pH varied from 4.95 to 6.17, a range of 1.22 pH. A significant correlation of −0.504 was calculated. He points out that there are undoubtedly other factors at play. Smith states in his conclusion, "The pH of 78 samples has been measured electrometrically. When these samples are divided into two groups according to soil type there is found to be a significant negative correlation between pH and cyst counts for 53 peat soils. There is not, however, a significant correlation in the case of the other 25 sandy soils."

The writers have been able to find no other citations referring to specific investigations on the influence of hydrogen-ion concentration of the soil on any phase of nematode biology. Emmert (8, p. 64) in connection with his extensive studies on effects of soil reaction on growth of tomatoes and lettuce, states in a brief paragraph his observation that extremes of acidity and alkalinity appeared to retard nematode development. Need for definite study on possible relationship between soil reaction and amount of soil infestation with *H. radicicola* is clearly indicated.

EXPERIMENTS

By way of preliminary experiment, the same method used by Burkholder (2), that of attempting to adjust soil pH by additions of sulfuric acid and of sodium hydroxide, was employed. The initial pH of a uniform lot of soil was first

taken and then, in different lots, calculated quantities of these two chemicals were added, and the soil was thoroughly mixed and allowed to stand for about a month. At this time hydrogen-ion determinations were made by the quinhydrone electrode method, and a pH range was obtained from pH 3.5 to pH 8.5, with intermediate points as shown in table 1. The soil was placed in root observation boxes (7) and pineapple slips were planted, two to a box, in May, 1928. Roots developed at the outset fairly satisfactorily in all cages, and on June 7, inoculations were made as follows: Suspensions of *H. radicicola* larvae containing approximately 10,000 larvae were poured about the roots of one plant only in a box, and on one side only of the box.

TABLE 1

Effect of differences in initial pH on infection by H. radicicola

NUMBER	pH	H. RADICICOLA INFECTION	CONDITION OF ROOTS
1	3.5	0	Very poor
2	3.5	6	Fair
3-C*	3.5	0	Fair
4	4.5	8	Fair
5-C	4 5	0	Good
6	6 5	8	Poor
7	6 5	8	Poor
8	6 5	10	Poor
9	7 0	10	Fair
10	7 0	10	Poor
11	7 0	10	Fair
12	7.0	10	Fair
13-C	7.0	0	Good
14	8 5	10	Poor
15	8 5	8	Fair
16	8 5	10	Poor
17	8.5	10	Poor
18-C	8.5	0	Good

* C, uninoculated controls.

Observations were made from time to time and it was noted that at least at the intermediate pH values infection took place at once and went through the usual stages of development of new generations and spread from one plant to another until the root systems were pretty generally infected. At the extremes both high and low, of soil pH, root growth was poor but definite infection was noted throughout. Very poor growth occurred at pH 3.5. At 4.5 it was good. At pH 8.5 growth was poor but somewhat better than at 3.5.

On December 19, 1928, about 6 months after inoculation, all plants were removed, the roots washed free of soil, and observations taken on extent of development of root knot. The scale used is an arbitrary one, and is useful only to record relative amounts. It may be considered, however, that the index number 10 means definite, uniform heavy infection and numbers less

than that correspondingly lighter infection. Unfortunately the press of other activities did not permit of checking the pH of the soil, either during the course of growth or at the close of the experiment. The hydrogen-ion concentrations recorded, therefore, are those made at planting time only.

It is obvious that infection occurred throughout the range of initial hydrogen-ion concentrations. If anything, it is more consistently heavy through the middle range, though this may have been due in part to the more consistent availability of susceptible roots.

This experiment, although unsatisfactory as to the reliability of hydrogen-ion concentrations during plant growth, is at least a strong indication that the root knot nematode is tolerant of a wide range of soil reactions.

TEST OF FIELD SOILS OF WIDE pH RANGE

It was well known that the sugar cane and pineapple field sof Hawaii consistently maintain their levels of hydrogen-ion concentration unless specifically treated to bring about a change, and that a very wide range was to be found in different localities. In the higher (mauka) cultivated areas where rainfall is high (up to 150 inches), and the virgin vegetation had been dense, soils occurred with a pH of 4.0 or even lower, while in the lower (makai) sugar cane field levels, especially near ancient coral beds, the pH was often around 8.0.

By consultation with plantation managers, and with the coöperation of their field men, it was possible to locate fields upon which pH readings had been taken from such locations and transported to the Wahiawa Field Station.

It was desired to bring about uniformity as to *H. radicicola* infestation, without too greatly altering the quality of the soils, as often results from heat sterilization. Consequently these soils were spread in thin layers to dry, in a sun exposure. It was known from previous studies (9) that this would bring about complete sterilization in so far as the root knot nematode was concerned.

The dried soils were then tested colorimetrically as to pH by Mr. Abel of this station and samples selected with a desirable range. These were moistened uniformly and placed in the same type of root observation boxes as those used before, there being six boxes for each soil selected. Here they were allowed to stand for 2 weeks when composite samples were made up of about 100 gm. of soil from each of the six boxes, and further pH determinations were made electrometrically at two separate laboratories, by the hydrogen electrode method. The determinations are listed in columns 2 and 3 of table 2. The boxes of each of the selected soils were now divided into two lots of three boxes each. One set was planted to cowpeas the other to pineapples. It should be recognized that the soils for this were collected from a very wide range of locations, consequently they differ widely in soil type and fertility. These differences obviously might be expected to influence plant growth, as they actually did. Records were made of such differences, in some cases photographically, but since they were not sufficient to alter the major conclusions that are justified with regard to the primary consideration, that of influence of soil hydrogen-ion concentration, they are omitted from this report.

Test with Whippoorwill cowpeas

On July 12, 1930, cowpeas were planted in the set of boxes segregated for the purpose. On July 18, after the seeds had germinated, inoculations with *H. radicicola* were made by placing 50 egg masses (potentially about 25,000 larvae) in a horizontal line in the soil on the face of the cage, about 5 inches below the soil surface. Inoculation with egg masses insured that all phases of nematode activity, hatching, migration through the soil, and actual infection of the roots, were subject to the influence of the hydrogen-ion concentration of the soil of the particular root observation box concerned. After the initial readings on soil pH, additional readings were taken at the time indicated in table 2. Such readings are included, as well as results in nematode infection, in the one table.

Preliminary pH readings were taken colorimetrically, mostly in the field, as a guide to selection of soils. The first and second readings here recorded, taken

TABLE 2

Effect of hydrogen-ion concentration on infection in cowpeas by H. radicicola

SOIL NO.	pH DETERMINATIONS					H. RADICICOLA INFECTION 9-18-30		
	Original (6-30-30)		8-4-30	Final 9-18-30	Average pH			
	E-1	E-2				A	B	C
6	4.1	4.1	4 1	4 3	4 2	7	10	10
5	4 4	4 3	4 3	4 2	4 25	10	9	10
2	4 5	4 4	4 4	4 4	4 4	9	8	10
4	4 9	4 7	4 7	4 7	4 7	10	10	9
10	6 1	6 3	6 0	6 2	6 1	10	10	10
1	6 6	6.6	6 4	6 3	6 35	9	10	9
16	7 8	7 8	7.8	7 6	7.7	10	10	7
11	8.0	7 8	7 8	7.7	7 75	9	8	9
14	8 1	8 0	7 9	7 7	7.8	8	8	8

after the soil was dried, constituted a check on a newly installed apparatus in our chemical laboratory. E-1 was an electrometric (hydrogen electrode) determination made, through the courtesy of Dr. F. E. Hance at the Experiment Station of the Hawaiian Sugar Planters' Association, on the soil suspension. E-2 was obtained by Dr. O. C. Magistad at the same time on the thick soil suspension in the A. H. P. C. laboratory with a hydrogen electrode. Subsequent readings were made in the A. H. P. C. laboratory by means of the hydrogen electrode. It is noteworthy that the readings remained fairly constant through the experiment, and that the original order of soils in hydrogen-ion concentration was not changed.

The final determinations of degree of root knot infection were made on the basis of relative values, as before, and can not be interpreted in terms of actual numbers of nematodes penetrating the roots. It was obvious that infection was almost uniformly heavy throughout, and a detailed count was not justified

in that differences would be insignificantly small. There was probably somewhat less infection at the highest pH values, 7.75 and 7.8, though there is no detectable difference between 4.2 and 7.7.

Inspection of the data in the table makes it apparent, without calculating, that the correlation between pH and degree of infection of plants is very low, if indeed not quite zero. This being the case, there can be no particular advantage in knowing the exact figure, as concluded by Smith (11) for his data on sandy soils. In order to make this report complete, however, such correlation was calculated, with 0.25-pH step intervals for the pH and unit index numbers for degree of nematode infection, and found to be -0.325 ± 0.115, a very low negative correlation, and an insignificant one, from the statistical point of view.

TABLE 3

Influence of hydrogen-ion concentration of soil on H. radicicola infection in pineapple

SOIL NO.	pH DETERMINATIONS		H. RADICICOLA (PER CENT ROOT INFECTION)		
	Original (8-14-30)	Final (12-12-30)	D	E	F
6	4 1	4 0	90	96	97
5	4.2	4.2	48	48	85
2	4 3	4 3	91	97	97
4	4 7	4 7	75	59	73
10	6.3	5.9	88	67	90
1	6 4	6.3	74	98	88
16	7.6	7.5	58	60	55
11	7.7	7.7	94	80	85
14	7.7	7.6	55	30	8

Test with Smooth Cayenne pineapple

In the duplicate set of soils, three boxes of each soil, plantings were made on July 14, 1930, two pineapple slips to each box. On August 13, the plants were well established, roots being 3 to 6 inches long. At this time *H. radicicola* was applied in the form of egg masses, approximately 50 to the cage (potentially 25,000 larvae) equally distributed between the two sides mostly at root tips but intermediately placed as well.

The initial pH of the soils was the same as in the cowpea test just described. Subsequent determinations were made by the hydrogen electrode method in A. H. P. C. chemical laboratory. They are listed, along with results in *H. radicicola* infection, in table 3.

Again, in the pineapple test, the soil hydrogen-ion concentration remained fairly consistent at the final determination, as compared with the one made shortly after planting. Those with the higher pH values dropped slightly.

The readings on nematode infection are recorded as percentage of main roots showing distinct galls. The results are not so consistent as those in cowpeas. The writers' interpretation of this lies in the inherent poorness of the pineapple

root system in indicating to any degree of accuracy minor differences in *H. radicicola* population during the first few months of growth. This conclusion is partly the result of observations conducted elsewhere than in this particular test. The difference between soils 11 and 14 in nematode infection may be due to soil differences other than soil pH, the latter being negligible. Soil 14 was a red silty clay loam, rather sticky, and probably unfavorable for nematode activity.

Irregular as are the results, they again show the possibility for nematode activity and reproduction through a rather wide range of pH values.

The correlation between pH of the soil and extent of nematode infection in pineapples was very low, as is evident from the table. As calculated, with 0.25 pH on the one hand and 10 per cent root infection on the other (the single wide variant of 8 per cent being disregarded), such correlation was -0.231 ± 0.121, definitely low, and definitely not significant.

DISCUSSION

The results in extent of nematode infection from both cowpea and pineapple tests, verify the conclusions from observation that the hydrogen-ion concentration of the soil is unimportant in determining amount of infection on the part of *H. radicicola*. Two methods of controlling soil pH were included in our tests. In the first, starting with a soil of known acidity, a range from pH 3.5 to pH 8.5 was obtained by the addition of H_2SO_4 and NaOH. In the second, field soils were selected in a range from pH 4.1 to pH 7.7. In both series, uniform heavy inoculations were made with *H. radicicola* egg masses. Since at both ends as well as intermediately on the scale infection was high, probably no adjustment of soil pH is justified from the point of view of reducing the amount of damage done to crops by this organism.

A radical difference between this conclusion and that drawn by both Peters (10) and Smith (11) for *H. schachtii* appears to merit discussion. Peters not having given a figure for coefficient of correlation with his data on the plots under study, one of the present writers considered it worth while to calculate such coefficient with 0.1 pH for the step interval on the one hand and 20 cysts on the other, using the formula:

$$r = \frac{\frac{\Sigma xy}{n} - cx\, cy}{\sigma x\, \sigma y}$$

He arrived at the figure $-.506 \pm .071$. This is a distinct negative correlation and apparently a significant one. The negative correlation is not a high one, however, and it would seem certain that some factor other than soil pH was concerned in the variations in cyst counts. The range of pH values, only 0.7 pH, would appear to be rather low for satisfactory conclusions.

Peters says (10, p. 112) "Once the eelworm had become established over the field it would presumably select the more acid environment as it appears to

have done in the Institute experimental field." By "select" in this case the writer undoubtedly meant not any activity on the part of any particular nematode, but rather the more rapid propagation of nematodes in the more favorable environment. Since, as Peters says, several generations of *H. schachtii* may develop in a single season, any slight difference in the suitability of the environment may account for considerable differences in cyst counts by the end of the season. It appears possible that a difference in host variety may have accounted for the differences that were noted. The two plots with the lowest cyst counts were of two varieties not appearing elsewhere in the table. It would appear that these particular varieties may have been somewhat resistant and may have been growing only by chance where the pH was somewhat higher than at other points. Opposed to this, of course, is Peters' statement that Morgan (who did the nematode work) could see no correlation between cyst counts and potato varieties (10, p. 108). Again, these plots may by chance have had a particularly low initial nematode population. That there must have been considerable irregularity in this respect is shown elsewhere in the data. With the variety "Ally," for example, in two separate plots which showed a difference in pH of less than 0.1, there was a difference of 76 to 43, or nearly 2 to 1, in cyst counts. Approximately the same sort of inconsistency was shown with the other variety that was duplicated in the chart.

It is to be noted that with these two varieties the individual plots with the lowest cyst counts were in one part of the field and those with the highest in another. This corresponds, of course, with the difference in pH as stated by the author. It is conceivable that it may also correspond, however, with a difference in some other factor, as for instance, moisture retention in the soil. Indeed, in the paper by Smith (11) it would appear from his figure 1, that in one of three series, an apparent negative correlation exists between moisture and cyst counts. In the other two series in this figure there was virtually no variation in the moisture content. The variability in cyst count shown may have been the normal variability within a single moisture content array. It is unfortunate that no other figures exist in the literature with a wide range of moistures in the same type of soil, by which one might judge as to the possible influence of this factor on cyst count. In Smith's paper again, only a narrow range of pH values (1.22 pH) is shown. His calculated correlation between pH within this range, and cyst count is $-.504$ with a P. E. (calculated by the present writers) of 0.069. The fact that this figure agrees very closely with that calculated on the data given by Peters, makes both appear more significant. The inconsistencies in the matter of correlation reported from other fields by both Peters and Smith, however, would seem to add weight to the possibility that soil moisture, or at least some factor other than soil pH, might have even more weight than hydrogen-ion concentration in determining the building up of populations of *H. schachtii*.

In considering further the apparent difference between *H. radicicola* and *H. schachtii* in the effect of the differences of soil pH it is to be remembered, of

course, that the latter organism lives the greater part of its life superficially on the root with its body in the soil. It is thus more directly subjected to the soil environment than is *H. radicicola* and therefore may be more greatly influenced by the hydrogen-ion concentration of the soil. The writers feel, however, that considerably more work is demanded before the final story is told for *H. schachtii.*

In verification of our conclusions from the experiments reported herein, are field observations on the occurrence of heavy *H. radicicola* infestations in both acid and alkaline soils. Such infestations are decidedly spotted at times. One spot about 10 by 20 feet in an acid soil district (pH around 4.0) showed very high infestation in its middle portion with evidences of spread with the slope of the land. Surrounding areas were free. Samples taken in this district showed, of course, extreme variation in nematode infestation with approximately the same pH throughout. On the other extreme, heavy infestations were likewise found in soils of pH around 7.5, decidedly on the alkaline side of neutrality.

SUMMARY

Tests were conducted in Hawaii with a range of hydrogen-ion concentrations between pH 4.0 and pH 8.5 to determine whether this factor of the soil environment could influence amount of damage done by the root knot nematode *Heterodera radicicola* (Greeff) Müller. These tests were conducted in two ways, (*a*) with soils adjusted to different pH values by means of additions of H_2SO_4 and NaOH, and (*b*) the collection of pineapple and sugar cane field soils of previously determined pH values, through a wide range. In the first case pineapple was used for determinations of amount of root knot development; in the second, both pineapple and cowpea were used. In no case was any great difference in amount of infection manifest. There is possibly a slight but unimportant reduction at pH values of 7.6 to 8.0 as compared with lower points.

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THE DURATION OF LIFE OF THE ROOT KNOT NEMATODE, *HETERODERA RADICICOLA*, IN SOILS SUBJECTED TO DRYING[1]

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Parallel with the laboratory studies conducted at this station on the relation of various factors of the environment of *Heterodera radicicola* eggs and larvae reported in other papers (5, 8), tests were conducted on this organism as it occurs in a soil environment. These studies had the objective in view of determining the duration of life of *H. radicicola* in soils subjected to various soil moisture conditions. The results have given a more complete understanding of what actually takes place in the field.

Very little specific information was found in the literature on this subject. The writers were encouraged in their work, however, by the results obtained in the laboratory phases of the study referred to. Certain statements in the literature were likewise encouraging as to the possibility of actually improving soil fertility by sun drying. Steenkamp (9) in particular has made some rather significant statements in this connection. He says, "The beneficial effect on fertility of drying and burning soil has long been known." Again, "The increased productivity caused by drying alluvial soil in direct sunlight has been fully recognized in India." Still further, "It is the purpose of this paper to throw more light on the problem of the increased fertility after drying." Opposed to this viewpoint, however, is the prevailing opinion as expressed by Watson (12) that long exposure of soil to sun drying "greatly impoverishes" it by destroying the humus as well as the bacteria and other organisms. It is certain, of course, that during the period of such exposure increase of organic matter by the growing of green manuring crops, which is often highly desirable, is prevented. Regardless of the merits of the fertility phases of the case, however, it is of interest to know, where root knot nematode infestation is a factor in plant growth, what the effect of exposure of infested soil to drying may be on this organism.

EXPERIMENTATION

Since each experiment described herein was slightly different from the one preceding, the various experiments are taken up separately and the specific

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methods followed are described for each. In all cases determinations on extent of nematode survival after the various treatments were made by the indicator plant method described elsewhere (4).

Experiment 1.—Effect of drying on survival of H. radicicola *in tubs of heavily infested soil*

In this preliminary test, soil from an infested field was further inoculated with nematodes contained in broken roots of infected pineapple plants. The soil was thoroughly mixed and placed in wooden tubs of 1,220 cubic inches capacity and 11-inch depth. These were then subjected to the following conditions:

Lot A. Kept continuously moist by daily addition of water.
Lot B. Allowed to dry without stirring. No moisture was added during the entire course of the experiment until time to make determination on extent of nematode survival.
Lot C. Allowed to become dry with the supplementary aid of stirring of the upper 4 or 5 inches of soil once a week.
Lot D. Hastened in its drying still further by stirring once a week to the entire depth of the soil.
Lot E. Still further hastened in its drying by completely reversing the soil by pouring from one tub to another once a week.

There were six tubs in each lot.

The tubs were set up on benches in an open-sided greenhouse which was protected from rain but permitted the free passage of air. Since the house was covered with ordinary greenhouse glass, there were no direct ultra-violet rays acting upon the surface of the soil. The test began on April 17, 1928, and continued through the summer months. The temperature at no time was high enough noticeably to injure plants growing in the greenhouse. Such mortality of nematodes as occurred was due, in all probability, to the direct effect of drying and to starvation.

At the beginning of the test, check tubs were planted to cowpeas as an indicator of primary degree of infestation of the soil. All plants showed extremely high infection—beyond the practicability of a count.

One month after the start of the series of special treatments, one tub from each of the five lots was irrigated to approximate field capacity and cowpeas were planted as indicators to measure the degree of soil infestation. Thereafter, at 4-week intervals the special treatments were discontinued on one tub from each lot, moisture was added, and cowpeas were planted. The plants were kept under good growing conditions. They were removed with roots intact after 4 weeks' growth, this being sufficient time for thorough primary infection without chance of secondary galls appearing as the result of reinfection from the progeny of the first. Readings on the degree of remaining infestation were taken in terms of approximate numbers of galls per tub. Instead of an exact count of galls, relative degrees of infestation are here recorded on the scale of

0 to 10, 0 meaning, of course, no infection; trace means 1 to 10 galls per plant; 1 means 11 to 30 galls; 2, 31 to 50 galls; 3, 51 to 100 galls; 4, 101 to 200 galls; 5, 201 to 300 galls; 6, 301 to 400 galls; and therafter jumps of 200 for each index number, up to 10 meaning 1,000 or more galls per plant. These figures are estimates only; they are not susceptible to satisfactory statistical analysis, yet they are definitely significant as to important differences in soil infestation. The number 7, for example, would mean marked infestation as compared with only light infestation where the index is 1 or 2.

Table 1 gives the results in the successive readings. It shows gradual decrease of effective nematode populations of the soil with the passage of time in all five lots. The decreases were considerably faster in those soils that were most completely dried. At the time of the fourth reading on soil treated for 16 weeks, there was 100 per cent killing in the two soils that were driest, but killing was not complete in any of the others.

TABLE 1

Observations on root knot indicator crops from experiment 1

Readings are on the scale of 1 to 10 (very slight to extreme infestation)

SERIES	TREATMENT	PERIOD OF TREATMENT				
		4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
		Cowpeas				Pineapple
A	Continuous moisture	10	7	2	2	trace
B	Dry—no stirring	10	9	7	6	3
C	Dry—stirred 5 inches	10	9	1	1	0
D	Dry—stirred to bottom	10	8	1	0	0
E	Dry—soil turned over	10	2	trace	0	0

The soil that was permitted to become dry without watering and without stirring interestingly enough retained a higher nematode population at the end of the sixteenth week than did the soil which was kept continuously moist during the same period. This appeared at first anomalous in view of the fact that laboratory tests reported elsewhere (5) had shown relatively quick killing of *Heterodera* eggs and larvae at almost any humidity below saturation. At 90 per cent humidity, for example, killing of eggs in egg masses was complete in only 9 hours. Where the inoculum was in the form of infested roots, however, survival was much longer. Killing then was complete in 8 days at 80 per cent humidity. At 90 per cent humidity, however, there was still 70 per cent survival after 20 days. In the present test inoculum was in the form of infested pineapple roots. It would seem that the heavy survival indicated, after a period of 16 weeks of exposure, is to be accounted for on the basis of failure of the infested material to become completely dry. This is directly in line with the observational evidence of Rotmistrov quoted by Veihmeyer (10) and the latter's experimental evidence to the effect that in the absence of roots of growing plants, soil below the immediate surface layer will not lose its moisture

except at the extremely slow rate induced by thermal effects. Conrad and Veihmeyer (2) still further show that the rate of drying of soil is directly proportional to the abundance of living roots present in the soil. In this experiment no plants were present. It is therefore to be expected that throughout the soil, except in the surface layer, sufficient moisture was retained in the soil particles to maintain a relative humidity of 100 per cent in the soil interspaces. Under such conditions the nematode larvae lack water in which to swim and are therefore inactive; but as shown by the laboratory tests (5) long continued survival of *Heterodera* larvae and eggs will occur in an environment of 100 per cent relative humidity, in the absence of free water.

While no actual measurements were made of the humidity of the soil in this particular test, observations by means of a Fuess soil hygrometer, made later in tubs of soil and in field soils that were continuously cultivated during a dry summer, showed that this explanation is justified. Even though the soil appears superficially to be quite dry, readings were invariably 100 per cent or very nearly so for soil air humidity at any depth below the upper 2 or 3 inches of actual dust. The nematodes in such soil in the absence of free water, remained inactive but did not become dried to the killing point.

In the continuously moist soil the amount of water added daily was, unfortunately, not measured. It is impossible, therefore, to know the exact moisture content of the soil in relation to its field capacity. It is certain, however, that an excess of water was added above its moisture equivalent content, for at the close of the experiment the soil was actually soggy in the bottoms of the tubs. A film of moisture was therefore present in which the nematode larvae could maintain continuous activity after having once hatched. This continuous activity brought about gradual depletion of stored energy in the form of layers of fat in the cells of the intestinal wall which we have demonstrated for *H. radicicola*, as Goodey (7) has done for many other species of nematodes. In the absence of food they gradually starved. This conclusion is more or less theoretical but is logical in view of observed facts. No effort was made to control the possible occurrence of other organisms that might possibly have been a factor in nematode reduction in the moist soil.

At the time of this fourth reading, after 16 weeks' continuous soil treatment, lots D and E, as stated, showed no infection in the indicator crops. The 16 weeks of severe drying had apparently destroyed all infective nematodes. The other tubs in the series all showed some degree of infestation of the soil varying from 10 or 20 galls to the tub in lot C, up to 300 to 400 galls to the tub in lot B. At the time of this reading the remaining two tubs in each lot had had 20 weeks of continuous treatment. At this time these tubs, together with those upon which readings had already been taken, were watered heavily and allowed to stand for a week. They were then planted to pineapples and maintained under good growing conditions for several months. The pineapple plants were then removed from each lot. Observations were made on the pineapple roots and recorded as follows: lots C, D and E showed no infesta-

tion whatever, verifying the previous observation on the cowpea indicator that the nematodes had been completely eradicated by the drying treatment; lots A and B showed infestation in approximately the same degree as shown on the cowpea plants, a trace only in the continuously moist tubs and somewhat heavier infestation in the continuously dry but not stirred series. The pineapple root readings are significant merely in that they verify the earlier indicator crop readings and therefore justify the conclusion as to survival of the nematodes under the different conditions of the experiment.

Experiment 2.—A test of the survival of H. radicicola *eggs and larvae in soils*

The plan of this experiment called for conducting exactly parallel tests with *H. radicicola* larvae and egg masses, under a series of different moisture conditions. The soil used was pineapple field soil supplemented by the addition of 10 per cent organic matter in the form of peat moss. It was thoroughly mixed, steam sterilized, and then placed into a series of 98 four-gallon stone jars, each jar receiving exactly 7 kgm. of field soil of uniform moisture content, which was determined at the time of filling as 20 per cent of the oven-dry weight. The moisture equivalent of the soil, which was taken as the standard for moisture capacity according to the method of Briggs and McLane (1) and of Veihmeyer, et al. (11), was found to be 33 per cent. The jars were divided into two equal lots of 49 jars each. In lot A each jar was inoculated with *H. radicicola* larvae by pouring into it a suspension of about 10 cc. containing approximately 20,000 larvae obtained as described in another paper (3). This was stirred loosely into the soil in order to get approximately even distribution. Lot B was inoculated by inserting 40 egg masses, which quantity is sufficient to make approximately the same degree of initial infestation as that used in the first lot. The egg masses likewise were mixed throughout with the contents of each jar. Each of lots A and B was divided further into seven sub-lots, seven jars to each. These received the following treatments:

Sublot 1, undisturbed, surface dried, no stirring, no watering.
Sublot 2, dried by thorough stirring once a week, no watering.
Sublot 3, fluctuating between moisture equivalent and the minimum moisture attained by complete stirring once a week for a month.
Sublot 4, moisture equivalent maintained by daily weighing and water replacement.
Sublot 4, moisture equivalent maintained by daily weighing and water replacement.
Sublot 5, moisture equivalent maintained by sealing top with paraffin.
Sublot 6, dried to 75 per cent of moisture equivalent; sealed with parafin.
Sublot 7, dried to 50 per cent of moisture equivalent; sealed with paraffin.
Aeration a factor in 5, 6, and 7.

The jars were arranged on tables in a closed greenhouse with relatively uniform lighting throughout.

According to plan the treatments were continued for eight consecutive weeks when one jar from each sublot was brought to moisture equivalent water content and planted to cowpeas as a nematode indicator. Thereafter at regular

4-week periods another jar was similarly planted from each sublot until the entire 7 jars were thus tested. Each planting was examined after about 30 days' growth for survival of the nematodes, as manifested by the development of root knot.

During the course of the experiment records were maintained of water losses in those series that were allowed to become dry. These losses are expressed in table 2 in terms of percentage of moisture equivalent. The figures in the last column are the "relative wetness" of Conrad and Veihmeyer (2), or the ratio of the moisture content to the moisture equivalent expressed in percentage, at the time of the last reading. These figures provide information as to the rate and extent of drying in the dried soils.

TABLE 2

Water losses from soils allowed to dry in experiment 2

SUBLOT NUMBER	LOSS AT DIFFERENT PERIODS, IN PER CENT OF MOISTURE EQUIVALENT						RELATIVE WETNESS REMAINING
	1st 4 wks.	2nd 4 wks.	3rd 4 wks.	4th 4 wks.	5th 4 wks.	Total 20 wks.	
1	45 5	5 2	4 2	1 3	0 5	56 7	43.3
2	65 0	3.9	1.56	+	+	72.0	28 0

TABLE 3

Survival period of H. radicicola larvae and eggs in experiment 2

SUBLOT NUMBER	TREATMENT	SURVIVAL PERIOD IN WEEKS	
		Lot A (larvae)	Lot B (eggs)
1	Dried, no stirring	20	12
2	Dried, stirred	16	16
3	Fluctuating	8	20
4	Moisture equivalent	20	28
5	Moisture equivalent water sealed	8	8
6	75 per cent moisture equivalent	8	8
7	50 per cent moisture equivalent	8	20

Certain unavoidable circumstances served to vitiate the results to some extent. These were primarily excessive temperatures reached in the closed greenhouse during the summer months (the temperature reached 45°C. for brief periods on some days) and the destruction of indicator plants by rats. The latter of circumstance undoubtedly killed plants that contained immature nematodes, consequently the replants could not give the same reading as the original would have done had it survived. The result is uncertainty as to the verity of negative readings. The positive readings are reliable, however, and are stated briefly below in table 3.

The survival periods indicated are those at which the last positive reading was obtained. It can be taken for granted that at the last positive reading,

the actual amount of infection, or the "index of infection" as shown in table 1, is very low, as a rule only a trace. Whenever such index was this low, invariably the next reading 4 weeks later, was zero. As stated before, these results were vitiated by unfavorable circumstances and must not be considered reliable as to negative readings. It is very possible that under better conditions longer survival might have obtained. For example in lot 1, longer survival of eggs is to be expected, since all other observations have indicated greater hardiness in eggs than in larvae. The positive readings are of interest, however. They show definite long-time survival of *H. radicicola* eggs and larvae in a soil environment, even in the case of those soils in which moisture was low. In general (see lots, 3, and 4, and 7) the jars in which the inoculation was in the form of egg masses showed longer survival of infestation than did those that were inoculated with larvae.

Those jars that were dried without stirring showed survival for 20 weeks, which is about the same as in experiment 1. The jars dried with complete stirring showed survival for 16 weeks, which is the period at which complete mortality occurred in the first experiment. The jars subjected to fluctuations of moisture content showed relatively quick killing of larvae and survival of inoculum in the form of egg masses for 20 weeks with complete killing at 24. Those maintained at moisture equivalent showed survival in the cases of initial egg inoculum for 28 weeks. This is 8 weeks longer than in the wet lot of experiment 1. It brings into question, under moisture conditions at or below the moisture equivalent, the presence or absence of a film of moisture about the soil particles in which a nematode is capable of maintaining activity. If such film does not exist, then conditions might be considered to be ideal for long survival of nematodes either in the larval or egg form. There is then no opportunity for continuous activity, though hatching of eggs may occur; with the complete absence of drying the nematodes might theoretically be expected to survive for a long period. In the first test unquestionably in the continuously moist soil, a surplus of water was present and opportunity existed for hatching and for continued activity.

Experiment 3.—A test of survival of H. radicicola *larvae and eggs free in the soil and enclosed within infested roots under different soil moisture conditions*

This test was conducted under conditions very similar to those described in experiment 2. The soil used was a uniform, thoroughly mixed field soil supplemented by the addition of 10 per cent organic matter, steam sterilized and placed in 4-gallon stone jars. Its moisture equivalent was 35 per cent, at which point it was adjusted at the start of the experiment. The test was conducted in an opensided greenhouse; consequently temperatures were never higher than 32°C. (86°F.) as shown by a continuous thermographic record. The higher points mentioned were reached for only an hour or two each on two consecutive weeks; consequently temperature cannot be considered a factor in the killing of nematodes as it might have been in the previous experiment.

There were three lots of jars inoculated as follows: lot A, with a suspension of *H. radicicola* larvae, about 20 thousand to the jar; lot B, with about 40 egg masses each (roughly equivalent in potential numbers to the larva inoculation); lot C, with heavily infested pineapple root knot material, comparable with the others in nematode content. Treatments were planned as follows for lots A, B, and C. Each sublot consisted of 10 jars.

Sublot 1, drying facilitated by stirring thoroughly once a week with a trowel.
Sublot 2, moisture controlled by stirring thoroughly once a week for a month to bring the moisture content down as low as possible, then restoring to the moisture equivalent, and repeating.
Sublot 3, soil weighed daily and lost water replaced, keeping constantly at or near the moisture equivalent.
Sublot 4, moisture equivalent maintained by sealing the top with a paraffin-wax mixture poured over paper cut to fit, to a depth of about one-quarter inch. Aeration a factor.

The experiment was started on September 15, 1930. The schedule of outlined treatments was followed closely. Occasionally difficulty was experienced

TABLE 4

Survival of H. radicicola larvae, eggs in egg masses, and nematodes enclosed within roots, in soils subjected to different moisture conditions

SUBLOT NUMBER	TREATMENT	H. RADICICOLA SURVIVAL—WEEKS		
		Lot A (larvae)	Lot B (eggs)	Lot C (roots)
1	Dried, by stirring	16	20	20
2	Fluctuating	12	16	40+
3	Moisture equivalent	40+	40+	40+
4	Moisture equivalent sealed	14	14	20+

in obtaining satisfactory growth of the cowpea indicator because of heavy attacks by leafhoppers. In general, however, satisfactory indicator crop readings on infection were obtained. Unfortunately the series of jars was not extensive enough to obtain a final negative reading on all treatments. It was not learned, therefore, at what time final killing might be expected under the conditions that were more favorable for nematode life. Under the less favorable conditions complete mortality occurred before the end of the experiment.

The results of this experiment are given in table 4 as the period of time elapsed up to the final readings which showed survival, all subsequent readings being negative. Where survival was still evident at the time of the readings on the final jars of the series, it is indicated by the symbol "+" meaning "more than."

Records which were maintained of water losses in sublot 1, dried, showed, at the time of the last positive readings, relative wetness as follows: lot A, larvae, 20.9 per cent; lot B, eggs, 14.8 per cent; lot C, roots, about the same.

It has seemed from this table that again complete air drying brought about

complete killing of all the nematodes in 20 to 24 weeks. This includes those in the relatively protected condition as found within woody infested roots. The fluctuating condition of alternate drying and wetting has apparently reduced eggs and larvae in this case even quicker than the drying alone. This condition, however, permitted survival of nematodes within the roots for 40 or more weeks. The lots maintained at the moisture equivalent, unsealed, permitted of survival for 40 or more weeks throughout. The jars with the same high moisture content sealed with the paraffin-wax mixture showed much quicker killing of the contained nematodes. Some condition, at present not thoroughly understood, is probably a factor here. Possibly decomposition of organic matter permitted the accumulation of toxic gases within the jars and this condition brought about a quicker killing. In general again it is to be seen that, as might have been expected, nematodes survived for a longer period when protected by the woody roots which contained them.

Experiment 4.—The duration of life of nematodes in pineapple roots

As a final experiment in this series, an effort was made to determine the period the root knot nematode would survive in pineapple roots exposed under various conditions. Heavily infected pineapple roots contain an abundant population of this organism. This is shown to good advantage by Godfrey and Oliveira (6) in their several illustrations. When such roots became buried in the soil they make a prolific source of infection for new plantings. The release of the nematodes into the soil may take place almost immediately in the case of the more tender root tip tissues, in which the larvae, hatching out from egg masses, may travel readily to reach the soil. Those galls that have become woody, however, which include most of the non-terminal galls and the very light infections not even evident as galls, serve as a medium in which the nematodes may survive for a long period. With such, actual root decay or partial decay must take place before their release into the soil can come about. The nematodes within such roots are more or less dormant populations, in egg or larval form. Such nematodes are likely to play an important part in the survival of infestation for a new planting. Doubtless, in the field, there are enormous numbers free in the soil, but all tests to date (5) have shown that those protected by the hardened root tissues have the highest degree of resistance to unfavorable conditions.

In June, 1929, a large number of heavily infected pineapple plants were selected from a field that was to be destroyed in the fall. Those selected were relatively uniform as to bulk and degree of nematode infection. In each case the top of the stump was cut off to prevent all possibility of growth, and the roots left attached to the base of the stump for convenient manipulation. The roots were thoroughly washed to free them of all surface individuals. They were then subjected to various treatments, as outlined in table 5. After the treatments, tests for survival were made by placing the root systems, still attached to the stumps, into gallon cans of moist sterilized soil in triplicate. Here they were left under moisture and temperature conditions favorable for

nematode activity for one week, to permit of escape of contained nematodes into the soil. They were then removed, washed thoroughly to free them from surface organisms, and placed in a second lot of sterile soil for a week, and so on through several changes. Meanwhile as each lot of soil was released, it was planted to cowpea as an indicator plant. One observation for nematode survival was made at the end of 30 days. The final reading, which can be considered as the completely reliable one, was made on the remaining two cans at the end of 60 days.

In table 5 the first column includes the treatment to which the roots were subjected; the second column, by plus and minus signs, the record as to survival of infestation after the treatment concerned; and the third, the number of transfers to new sterile cans before negative readings were obtained, or in other words, the number of weeks survival of nematodes actually within the roots. This latter reading can, perhaps, be looked upon as a rough measure of the magnitude of the infestation left surviving, after the drying treatment.

TABLE 5

The survival of nematodes in infested pineapple roots

INITIAL TREATMENT	SURVIVAL AFTER TREATMENT	SURVIVAL IN WEEKS WITHIN ROOTS IN MOIST SOIL, THEREAFTER
Placed immediately in moist soil	+	9
Roots dried under July sun 2 days	+	5
Roots dried under July sun 4 days	+	4
Roots dried under July sun 6 days	+	2
Roots dried under July sun 8 days	−	0

The conclusions to be drawn from this test are that (*a*) *H. radicicola* is capable of surviving within woody roots for at least 9 weeks of conditions which favor activity, before being completely released into the real soil environment. This period may, of course, vary widely from the figure given, depending on the rapidity of decay processes and other factors; (*b*) exposure of roots to sunshine for a few days prior to plowing them in may eliminate this source of infection completely, in so far as the new planting is concerned.

SUMMARY

Following an exhaustive study of effects of various controlled environmental factors, particularly heat, moisture, and light, on the survival of *H. radicicola* larvae and eggs, reported in a previous paper, studies were made on the duration of life of the same nematode in soils subjected to drying. Three experiments on soil heavily inoculated with this organism in various conditions in containers of about 20-liter (5-gallon) capacity, gave results as follows:

When the inoculum was in the form of heavily infested woody pineapple roots, life persisted, in soil that was dried, without stirring, for something over 20 weeks; in soil air dried with frequent stirring to hasten the drying, for 16 to 20 weeks or slightly more; in soil kept continuously wet, i.e. with an excess of moisture over "moisture equivalent' requirements,

for something over 20 weeks but to a much reduced extent, as compared with surface-dried soil; in soil kept continuously at moisture equivalent, for over 40 weeks.

When inoculum was in the form of *larvae* life persisted in surface dried soil, without stirring, for 20 to 24 weeks; in air-dried, frequently stirred soil, for 16 to 20 weeks; in soil kept continuously at moisture equivalent, for over 40 weeks; in soil fluctuating between moisture equivalent and air-dry conditions, for 12 to 16 weeks; in moisture equivalent soil kept sealed, which permitted the accumulation of decomposition gases, for 14 to 16 weeks.

Inoculum in the form of *egg masses* persisted as a rule for a period somewhat longer than when in the form of larvae at the outset.

Excess moisture, which provides a medium in which larvae may be continuously active, appears to hasten the exhaustion of their vitality (survival 20–24 weeks) as compared with soil in which high air humidity is maintained without the opportunity for continuous activity (survival 40 weeks +). The range of soil moisture contents in which this latter condition exists appears to be high, all the way, in "relative wetness," from about 50 per cent to 100 per cent.

The fluctuating condition between a slight excess of moisture and the air-dry condition, materially hastens the death of the organisms in egg and larval stages but not obviously so during the period of these experiments where they are protected by being embedded within woody roots.

Sealing the jars with a relatively high moisture content, which permits of continuous decomposition of contained organic matter, likewise hastens the death of the contained nematodes.

The root knot nematode may survive within woody roots for 9 weeks or longer, in a moist soil environment, and thus serve as a source of infection in subsequent plantings; such source of infection may be eliminated by exposure of such roots to the sun for a few days before they are plowed into the ground.

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ASSIMILATION OF PHOSPHORUS AND POTASSIUM BY BARLEY PLANTS GROWN ACCORDING TO NEUBAUER PROCEDURE AND IN UNDILUTED SOIL

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Among the numerous procedures used in attempting to ascertain the requirements of soils for plant nutrients, is that proposed by Neubauer (5, 6, 7). The essential feature of this method is the growing of a large number of rye plants in a small amount of soil mixed with sand. The quantities of phosphorus and potassium obtained from the soil indicate the available supply. This method has been used extensively in Germany where it is considered useful for ascertaining soil deficiencies. Roemer (8) applied the method to the continuously cropped soils from plots at Rothamsted and concluded that it was well adapted for determining the available plant food supply of the soil as influenced by fertilizer treatment and cropping.

In addition to the nutrient supply there are many environmental factors, including variations in temperature and moisture, that can influence the intake of nutrients by plants. Other factors that may affect the indications obtained by the plant's analysis of the soil, are the amount of soil and the influence of mixing sand with soil upon the feeding capacity of the root system of plants grown in pot tests. With conditions for root penetration and development improved there may be larger amounts of elements taken up than without sand addition.

Gericke (4) in a discussion of methods used for determining the soil supply of nutrients available for plants, directs attention to the influence of sand addition to soil in the seedling plant method of Neubauer. He states that the addition of a large amount of sand to a small amount of soil may modify the mechanical composition of the soil to the extent that its inherent individuality with respect to physical characteristics will be partially eliminated, and soils of different types therefore reduced to a common basis. This influence of sand is less noticeable in sandy and loam soils since there is sufficient opportunity for root penetration in these soils. The effect of sand addition in the case of soil of high clay content may be considerable. Under certain conditions without sand addition, the intake of root-soluble nutrients will be less than when soil is changed from its natural field condition by the addition of sand.

EXPERIMENTAL

One of the advantages claimed for the Neubauer method is that the extreme condition of competition with a large number of plants grown in a small amount of soil obscures the effect of other factors than nutrient supply to a considerable extent. The effect of a small amount of soil diluted with sand as compared with a larger amount of undiluted soil on the intake of phosphorus and potassium by plants is shown by results obtained with the plant analysis method in determining available nutrients in soil from several plots of the 5-year rotation fertility experiment at the Ohio Agricultural Experiment Station.

Soils and treatment

The soils were from plots of the 5-year rotation fertility experiments that have had additions of phosphorus supplied by superphosphate, bone meal, and basic slag supplemented with potassium and nitrogen on limed and unlimed soil. These phosphorus carriers have been applied to certain plots of this fertility experiment for 37 years. Crop yields on the limed portions of the plots in former years have exceeded those on unlimed soil. During recent years, however, the yields of oats and wheat on the limed portions of the plot treated with bone meal have tended to fall below the yields on the unlimed soil. Tests with barley plants grown in the two amounts of soils were employed for the purpose of determining whether a deficiency of available phosphorus or potassium was a factor producing this peculiar effect on crop yields following the long continued addition of bone meal as compared with other phosphorus carriers on limed soil.

The field applications of fertilizers that have been made on the plots described are stated in the tables giving the data pertaining to phosphorus and potassium obtained by plants from the differently treated soils. During the 27-year period preceding 1921 the fertilizer treatment for each 5-year period supplied phosphorus equivalent to the amount furnished by 16 per cent superphosphate at the rate of 320 pounds an acre. Since 1921 the phosphorus carriers have furnished phosphorus equivalent to 480 pounds of superphosphate. Muriate of potash has been applied at the rate of 260 pounds and nitrate of soda 320 pounds an acre each 5 years during the entire period.

The additions to soils in pots previous to the planting of barley were at the following rates per 2,000,000 pounds of soil considered as the weight per acre; superphosphate 20 per cent P_2O_5, 500 pounds; muriate of potash, 400 pounds; nitrate of soda, 125 pounds; calcium carbonate, 8,000 pounds. These additions to soil in pots were for the purpose of determining what effect modifying the soil's supply of constituents furnished by these treatments might have on the growth of plants as well as on their composition.

Methods

In applying the plant analyses method for determining the assimilable phosphorus and potassium in soil from these differently treated fertility plots,

100 barley plants were grown in 100 gm. of soil with addition of sand as prescribed by Neubauer, and in 9 pounds of undiluted soil. These were triplicate pots of each soil and treatment, and the growing period of plants in both amounts of soil was 18 days. The roots were discarded and portions of the plants above ground were used for determining the intake of phosphorus and potassium. The plants of both series were grown at the same time in a glass house with identical conditions of temperature and light, and the soils maintained at the same moisture content of 20 per cent.

PHOSPHORUS CONTENT OF PLANTS

A comparison of the phosphorus concentration or percentage in plants shows that this was higher in the barley from 100 gm. of soil as used in the Neubauer procedure. In fact the percentage of phosphorus in plants from the smaller amount of soil has rather uniformly exceeded that in plants from 9 pounds of undiluted soil by a wide margin. The percentages of phosphorus in barley plants grown under the different conditions of treatment are stated in table 1.

The larger weight of plant material generally produced in 9 pounds of soil was no doubt a factor partially accountable for the decreased phosphorus content of plants from the larger amount of soil as compared with that of the plants grown according to the Neubauer procedure. It will be noted, however, from the potassium data for plants in table 3 that the relation between weights of plants produced and their potassium content was directly opposite to that for phosphorus.

Effect of treatment on phosphorus content of plants

Considered in detail with respect to the field treatment of the soil with fertilizers and lime, and additions to pots, the variations in phosphorus content of plants from the two amounts of soil are as follows: With no additions of superphosphate or calcium carbonate to soil in pots, the previous field applications of lime materials has caused some variations in the phosphorus content or percentages in plants grown in both quantities of soil. The averages for the phosphorus content of plants grown in unlimed and limed soil, without addition of superphosphate to the pots, shows that there was a general tendency for the percentage in plants from the limed soil to be less than that of plants from unlimed soil in the Neubauer series. In the case of barley grown in 9 pounds of soil the plants from limed soil had a higher phosphorus content. With available phosphorus supplied to plants by additions of superphosphate when barley was planted there was generally an increased content of phosphorus in plants grown in both amounts of soil. This increase, however, was more pronounced in plants from the larger quantity of soil.

In the case of plants grown according to the Neubauer procedure the available phosphorus supplied by superphosphate was reflected to a greater extent by an increased percentage of phosphorus in plants grown in the limed soil. No evidence of an increased supply of phosphorus resulting from previous field applications of phosphates was furnished by the phosphorus content of plants.

TABLE 1

Phosphorus assimilation by barley plants grown in 100 gm. of soil according to the Neubauer procedure and in 9 pounds of soil

FIELD TREATMENT 5-YEAR ROTATION FERTILITY EXPERIMENT PLOTS	Additions to soil in pots*	NEUBAUER 100 GM. SOIL					9 POUNDS UNDILUTED SOIL				
		Plant weight	Phosphorus content of plants	Phosphorus balance			Plant weight	Phosphorus content of plants	Phosphorus balance		
				Phosphorus in soil†		Phosphorus removal by plants			Phosphorus in soil†		Phosphorus removal by plants
				Total	0.1 *N* H_2SO_4-soluble				Total	0.1 *N* H_2SO_4-soluble	
		gm.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*gm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*
Unlimed soil											
None	None	2.9	0.56	37	2	17	4.8	0.20	1,508	94	10
	$CaCO_3$	3.1	0.42	37	2	13	4.7	0.22	1,508	94	11
	P	2.9	0.61	40	5	18	4.0	0.36	1,599	183	14
	P, N	3.5	0.51	40	5	18	7.6	0.42	1,599	183	32
Superphosphate, muriate of potash, nitrate of soda	None	3.2	0.54	47	3	18	7.8	0.19	1,898	126	15
	$CaCO_3$	3.3	0.57	47	3	19	7.8	0.24	1,898	126	19
	P	3.2	0.47	50	6	15	8.2	0.37	1,989	215	30
Bone meal, muriate of potash, nitrate of soda	None	2.9	0.53	47	4	15	6.1	0.20	1,898	147	12
	$CaCO_3$	2.9	0.49	47	4	14	6.5	0.18	1,898	147	12
	P	2.8	0.64	50	7	18	7.0	0.35	1,989	236	24
	K	3.1	0.46	47	4	14	6.8	0.21	1,898	147	14
	N	2.9	0.46	47	4	13	6.2	0.19	1,898	147	12
Basic slag, muriate of potash, nitrate of soda	None	2.9	0.55	41	4	16	7.1	0.20	1,667	159	14
	P	3.2	0.54	44	7	17	8.4	0.44	1,758	248	37
Limed soil											
None	None	3.2	0.51	38	3	16	4.3	0.24	1,545	126	10
	P	3.3	0.68	41	6	23	7.1	0.47	1,636	215	33
	P, N	3.5	0.69	41	6	24	8.0	0.45	1,636	215	36
Superphosphate, muriate of potash, nitrate of soda	None	3.5	0.52	43	5	18	7.0	0.23	1,744	208	16
	P	3.4	0.73	46	8	25	8.4	0.38	1,835	297	32
Bone meal, muriate of potash, nitrate of soda	None	3.2	0.38	42	7	12	6.8	0.26	1,717	269	18
	P	3.4	0.56	45	10	19	7.5	0.46	1,808	358	34
	K	3.6	0.45	42	7	16	9.0	0.27	1,717	269	25
Basic slag, muriate of potash, nitrate of soda	None	3.5	0.53	41	5	19	6.2	0.23	1,658	192	14
	P	3.3	0.56	44	8	19	8.9	0.41	1,749	281	37

* P = superphosphate; K = muriate of potash; N = nitrate of soda.

† Plus amount supplied by pot additions of superphosphate.

Application of chemical methods to the soils from these several plots has furnished indications of variations in both the total and 0.1 *N* H_2SO_4-soluble phosphorus in these soils. Table 2 shows the increases of both forms of phosphorus in soil from unlimed and limed portions of plots fertilized with superphosphate, bone meal, and basic slag as compared with unfertilized soil. The percentage of the total phosphorus soluble in the acid used for extraction is also stated. There are larger amounts of both total and soluble phosphorus in soil from the phosphated plots, and there is no wide variation in the proportion of the total amount that was soluble in dilute acid, although this is somewhat more for the limed soil. Calculated on the basis of percentage increase over

TABLE 2

Total and 0.1 NH_2SO_4-soluble phosphorus

Pounds per acre 2,000,000 pounds of soil

PLOT TREATMENT	PHOSPHORUS		PER CENT OF TOTAL PHOSPHORUS SOLUBLE	PER CENT INCREASE OVER UNFERTILIZED SOIL	
	Total	Soluble		Total phosphorus	Soluble phosphorus
	pounds	*pounds*	*per cent*	*per cent*	*per cent*
Unlimed soil					
Unfertilized	750	46	6	. .	. . .
Superphosphate	929	62	7	24	35
Bone meal	927	72	8	24	57
Basic slag	819	78	10	9	70
Limed soil					
Unfertilized	809	63	8	. .	. .
Superphosphate	856	102	12	6	62
Bone meal	834	132	16	3	110
Basic slag	813	94	12	0.5	49

the unfertilized soil, the increased content of soluble phosphorus exceeds that for total phosphorus in soil from the phosphated plots.

Relation between soil phosphorus supply and removal by plants

In considering the relation of the amount of phosphorus in the soil to removal by plants, one could infer that the removal should be greater with an increased supply at the disposal of plant roots in 9 pounds of soil. The fact is, however, that this did not generally occur except in the case of plants from soil with readily available phosphorus supplied by superphosphate just before planting. A factor that may have had an appreciable influence in this connection, is the relatively greater proportion of phosphorus supplied in 100 gm. of soil by the barley seed. The barley seed contained 0.461 per cent of phosphorus and 0.547 per cent potassium. Calculated on the basis of 3.76

TABLE 3

Potassium assimilation by barley plants grown in 100 gm. of soil according to the Neubauer procedure and in 9 pounds of soil

FIELD TREATMENT 5-YEAR ROTATION FERTILITY EXPERIMENT PLOTS	Additions to soil in pots*	NEUBAUER 100 GM. SOIL				9 POUNDS UNDILUTED SOIL			
		Plant weight	Potassium content of plants	Potassium balance: Exchangeable potassium in soil†	Potassium balance: Potassium removal by plants	Plant weight	Potassium content of plants	Potassium balance: Exchangeable potassium in soil†	Potassium balance: Potassium removal by plants
		gm.	*per cent*	*mgm.*	*mgm.*	*gm.*	*per cent*	*mgm.*	*mgm.*
Unlimed soil									
None	None	2.9	1.28	3.9	38	4.8	2.72	159	131
	$CaCO_3$	3.1	1.50	3.9	46	4.7	2.20	159	103
	P	2.9	1.46	3.9	43	4.0	2.15	159	86
	P, N	3.5	1.18	3.9	41	7.6	2.78	159	211
Superphosphate, muriate of potash, nitrate of soda	None	3.2	1.99	9.8	65	7.8	4.35	400	339
	$CaCO_3$	3.3	1.78	9.8	58	7 8	3.87	400	302
	P	3.2	2.17	9.8	69	8.2	3.45	400	283
Bone meal, muriate of potash, nitrate of soda	None	2 9	2.32	7.8	66	6.1	4.75	318	290
	$CaCO_3$	2.9	2.27	7.8	66	6.5	3 97	318	258
	P	2.8	2 48	7.8	68	6.9	3.95	318	273
	K	3.1	3.88	18.0	120	6.8	5.83	763	396
	N	2.9	2.03	7.8	59	6.2	4.40	318	273
Basic slag, muriate of potash, nitrate of soda	None	2 9	1.58	9 8	45	7.1	3 26	400	231
	P	3.2	1.56	9 8	51	8.4	3.35	400	281
Limed soil									
None	None	3.2	1.13	3 5	37	4.3	1.70	143	73
	P	3 3	1 38	3.5	46	7.1	1.40	143	99
	P, N	3.5	1.16	3 5	40	8.0	1.48	143	118
Superphosphate, muriate of potash, nitrate of soda	None	3 5	1.44	5 5	50	7.0	3.21	224	225
	P	3.4	1.57	5.5	53	8.4	3.21	224	271
Bone meal, muriate of potash, nitrate of soda	None	3.2	1.46	5.1	46	6 8	2 22	208	151
	P	3.4	1.42	5.1	48	7.5	2.43	208	182
	K	3 6	3.38	15 3	123	9.0	2.52	653	227
Basic slag, muriate of potash, nitrate of soda	None	3.5	1.32	5.5	46	6.2	2.82	224	175
	P	3 3	1.34	5.5	44	8.9	2.65	224	236

* P = superphosphate; K = muriate of potash; N = nitrate of soda.

† Plus potassium supplied to pots by muriate of potash.

gm. as the weight of 100 seeds there was 17 mgm. of phosphorus that could be drawn on by plants. The intake of phosphorus from 100 seeds by barley plants grown in sand was 6 mgm. in the tops and 9 mgm. in the roots. This addition by the seed to 9 pounds of soil is probably not significant, but possibly may not be a negligible increase to the phosphorus supply in 100 gm. of soil. Dreyspring (2), in a discussion of the phosphorus content of soils of different countries, directs attention to the phosphorus supplied by the seed. He states that 100 rye grains for instance contain about 20 mgm. of phosphoric acid, whereas a soil very poor in phosphoric acid yielded only a few tenths of a milligram of phosphoric acid to the germinating plants.

The addition of superphosphate to the soil in pots supplied 3 mgm. of phosphorus to 100 gm. of soil, and 89 mgm. to 9 pounds of soil. The removal by germinating plants in the Neubauer soil medium was from 30 to 50 per cent of the total phosphorus supply of the soil, but not more than 2 per cent of the phosphorus supply was removed from 9 pounds of soil. If the phosphorus supply of the soil is considered on the basis of the amount soluble in 0.1 *N* H_2SO_4, the removal by plants in the Neubauer method was considerably in excess of the soil supply, whereas the plants grown in 9 pounds of soil removed not more than 17 per cent of the amount in the soil.

In the case of 9 pounds of soil there is a wide margin between the soil supply of total phosphorus and the amount in 100 barley seeds, but in 100 gm. of soil the seed supply of phosphorus more nearly approaches that of the soil. Dreyspring (3) found that on an average about 4 plant food units of assimilable phosphoric acid must be present in the soil in order that 1 unit may be assimilated by the plant during the first year of vegetation. In other words the plant during the first year of vegetative growth is able to assimilate only about 25 per cent of the root soluble phosphoric acid in the soil.

POTASSIUM CONTENT OF PLANTS

A reversal from the phosphorus assimilation by plants occurred in the case of potassium intake from the two quantities of soil in which 100 barley plants were grown. For in all instances the percentage of potassium in plants from the larger amount of soil exceeded to a considerable extent that in plants grown in 100 gm. of soil used in the Neubauer procedure. Table 3 shows the potassium content of plants. A more extensive root system developed by plants in the larger amount of soil may have been a factor contributing to the increased amount of potassium obtained from 9 pounds of soil. Bartholmew and Jansen (1), in an extensive study of absorption of potassium by plants, found that increased root development caused a greater feeding upon the replaceable potassium of soil.

Effect of soil treatment on potassium content of plants

There is a close correlation between the potassium content of the plants and the fertilizer treatment of the soil. The increased percentage of potassium in

plants grown according to the Neubauer procedure and in the larger amount of soil shows that there is more assimilable potassium in the soil from plots that had potassium included in the fertilizer treatment. Addition of available potassium when barley was planted produced a further increase in the potassium content of plants on these soils fertilized with potassium.

The lower percentage of potassium in plants grown on limed as compared with unlimed soil from several differently fertilized plots indicates that the reaction of the soil or its supply of calcium as modified by field applications of limestone has affected the capacity of the plants to assimilate potassium. This effect on the intake of potassium by plants grown in both quantities of soil in these experiments is in accord with the interrelation of potassium and calcium frequently found in investigations pertaining to absorption of ions by plants grown in soil and solution cultures. Tyson (9), in a study of the influence of soil conditions and fertilizer treatment upon the chemical composition of sugar beets, found that the application of lime caused a decrease in the potassium content of both leaves and roots.

Another noticeable difference in the potassium content of plants as influenced by the previous fertilizer treatment of the soil, is the decreased percentage in plants grown in soil from the limed as compared with the unlimed portion of the plot fertilized with bone meal. This condition of the potassium supply indicated by the plants' analysis of the soil may be a factor affecting the crop yields on the limed soil of this plot.

Relation between the potassium supply and removal by plants

The soil supply of replaceable or exchange potassium and the amounts removed by plants from the two amounts of soil are stated in table 3. Where the relation between the potassium supply and removal by plants is considered, the amount in soil is based on the replaceable potassium. This, of course, is not on a comparable basis with the total phosphorus supply but is more so with the soil phosphorus soluble in 0.1 *N* acid.

The soil from the plot that has not had any fertilizer treatment contained 3.9 mgm. of exchangeable potassium. Plants grown in sand obtained only 9 mgm. of potassium from 100 seeds that contained 21 mgm. Whether this was the amount that was obtained from the seed by plants when grown in soil with addition of sand and in undiluted soil is not known. Assuming that 9 mgm. was obtained from the seed, the plants grown in 100 gm. of unfertilized soil without any additions to pots obtained 25 mgm. in excess of the total exchangeable potassium and that obtained from the seed.

The 100 gm. of unlimed soil from the plot that had potassium furnished by the fertilizer treatment contained 7.8 mgm. of exchangeable potassium. The increased removal over this amount and that obtained from the seed by plants, excluding roots was 48 mgm. With 10 mgm. of available potassium supplied to 100 gm. of this soil by muriate of potash when barley was planted, the increase over the average removal by plants from the same fertilized soil with-

out further addition of potassium was 55 mgm. for the unlimed and 74 mgm. for the limed soil. A similar comparison for the removal from 9 pounds of unlimed soil shows an increase of 123 mgm., which is only 25 per cent of the 445 mgm. of available potassium added by muriate of potash.

The intake by barley plants from 100 gm. of the Wooster silt loam soil without addition of potassium when barley was planted shows that the removal was 21 mgm. more from soil previously fertilized with potassium than from unfertilized soil. Considering that 1 acre of this soil to a depth of 7 inches weighs approximately 2,000,000 pounds, an assimilation by plants of 1 mgm. of potassium from 100 gm. of soil corresponds to about 20 pounds of potassium per acre. Therefore the increased removal of 21 mgm. of root-soluble potassium by plants from 100 gm. of soil fertilized with potassium corresponds to 420 pounds of available potassium per acre.

The ratio of increased potassium in fertilized soil, as determined by chemical analysis, to the amount indicated by increased root solubility or analyses by the plant is about 1 to 3.5. There was a small increased weight of plants from potassium fertilized as compared with unfertilized soil, but there was a close relationship between the percentage or concentration of potassium in the plants and the amount removed from the soil, the magnitude of the former having affected the magnitude of the latter.

Removal by Barley Plants and Neubauer's Limiting Values for Root-Soluble Phosphorus and Potassium

According to Neubauer the following amounts of phosphorus and potassium in readily available form should be contained in 100 gm. of soil in order to produce high yields of different crops without application of fertilizers.

	Neubauer's Values	
	mgm. Phosphorus	*mgm. Potassium*
Barley and straw	3	12
Oats and straw	4	14
Wheat and straw	4	13
Rye and straw	4	14
Red clover hay	4	21
Potatoes with tops and leaves	4	30

Larger amounts of phosphorus and potassium than these limiting values were obtained by barley plants from Wooster silt loam soil. Deducting the removal of 6 mgm. of phosphorus obtained from seed by plants grown in sand, the average removal of phosphorus from 100 gm. of soil that had no addition of available phosphorus when barley was planted, is 10 mgm. The potassium removal from unfertilized soil by plants, less the amount from seed, was 27 mgm. In the case of soils that had previous fertilizer application of potassium there is a much wider margin between Neubauer's figures and the amounts of potassium obtained by barley plants. As the phosphorus assimilation by

barley plants does not indicate a deficiency of available phosphorus the results obtained by the plants analysis of the soil are not in agreement with crop response to phosphates on this soil. The results for potassium are more closely in accord with the field experiments with fertilizers.

SUMMARY

Plants grown according to the Neubauer procedure in a small quantity of soil and in a larger amount of undiluted soil furnished different indications regarding the phosphorus and potassium supply in soils of fertility experiment plots.

In this comparison of plants' intake of nutrients from two amounts of soil, 100 barley plants were grown for 18 days in 100 gm. of soil diluted with 300 gm. of pure quartz sand and in 9 pounds of undiluted soil.

The intake of phosphorus and potassium was influenced by the quantity of soil. There is an inverse relation between the percentage content of phosphorus and potassium in plants and the amount of soil. The plants grown in 100 gm. of soil contained more phosphorus whereas the potassium content was considerably greater in plants grown in the larger amount of soil.

Application of the plant analysis method for determining the available phosphorus supply in soils of fertility experiment plots did not furnish significant indications of increased amounts of phosphorus residual from superphosphate, bone meal, and basic slag. Available phosphorus supplied by superphosphate when the barley was planted was more consistently indicated by the phosphorus content of plants and removal from the larger amount of soil.

Both the Neubauer seedling procedure and the method of growing plants for the same length of time in undiluted soil as generally followed in vegetative tests showed there was a reserve supply of potassium in soil fertilized with muriate of potash.

According to the results obtained in this investigation, the plant analysis method proved to be satisfactory for furnishing information regarding the available potassium reserves from previous fertilizer treatment but failed to indicate differences in the phosphorus supply of unfertilized and phosphated soils.

Apparently barley grown in 100 gm. of soil according to the Neubauer method had the capacity to assimilate considerable potassium from other forms than that in exchangeable combination.

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INFLUENCE OF ORGANIC MATTER ON CROP YIELD AND ON CARBON-NITROGEN RATIO AND NITRATE FORMATION IN THE SOIL[1]

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In an earlier paper (1) it was shown that under some conditions organic matter, in large amounts, greatly depressed non-legume crop yields. For legumes the yields were about the same on the check cylinders as on those that received heavy applications of organic matter. The difference seems to be due to the fact that the large supply of organic matter results in the rapid increase of soil microörganisms and that these in turn compete with the plant for the nitrogen which is in the soil and in the applied organic matter. In this connection Russell (3, p. 371) says:

> The addition of plant residues to the soil is a normal occurrence and a recognised method of manuring land. The effects produced depend on the proportions of carbohydrate and protein present in the residues. If the conditions are favourable to the activity of micro-organisms the result of the addition is to cause an increase in numbers and in activity of the microorganisms: this is shown by increases in oxygen absorption and CO_2 evolution.

He explains that if the material is rich in energy supply but not in nitrogen the organisms may assimilate nitrate or ammonia already existing in the soil and thus reduce the amounts of these substances present.

It is just this condition which obtains in the cylinder soils to which the rye straw has been added. With large applications of organic matter the competition becomes so keen that the non-legume plant often suffers and the yield is reduced. On the other hand, the legume, if inoculated, has an unlimited reserve of nitrogen and therefore is not disturbed in its growth by the greatly increased microörganic population which robs the non-legume of its supply of nitrogen.

The work of the first 3 years was reported in the aforementioned paper. It seemed desirable, however, to continue the work for the purpose of noting (*a*) the effect of heavy applications of a mineral nitrogenous fertilizer on the yield, where the applications of organic matter were heavy, and (*b*) the effect of the organic matter on the carbon-nitrogen ratio, total nitrogen, and nitrate nitrogen of the soil.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

As in the previous experiment the work was conducted in cylinders with the following special treatment for each series:

Two cylinders with no organic matter
Two cylinders with rye straw cut fine, 1 ton an acre
Two cylinders with rye straw cut fine, 2 tons an acre
Two cylinders with rye straw cut fine, 4 tons an acre
Two cylinders with rye straw cut fine, 8 tons an acre

The straw was worked into the soil to the depth of 4 or 5 inches just before planting. Each cylinder received 20 gm. of superphosphate (640 pounds an acre) and 10 gm. of muriate of potash. The standard application of nitrate of soda was 10 gm. for a cylinder, but in certain cases two and even three times

TABLE 1

Yields of dry matter from soybeans, barley, and oats—1924–1931

CYLINDER NO. AND DATE / SPECIAL TREATMENT (ACRE BASIS)	SOYBEANS					BARLEY					OATS				TOTAL BARLEY AND OATS
	121–130 1924	131–140 1924	121–130 1925	131–140 1925	Total	141–150 1924	141–150 1925	141–150 1926	141–150* 1930	151–160† 1930	141–150† 1927	151–160* 1927	121–130‡ 1931	131–140† 1931	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
No rye straw	176	279	73	124	652	137	72	77	77	67	43	98	158	38	767
1 ton rye straw	201	289	107	125	722	132	73	67	40	32	41	98	177	29	689
2 tons rye straw	203	288	120	143	754	118	92	81	64	31	40	79	157	35	697
4 tons rye straw	167	190	79	125	561	75	84	61	64	36	29	54	164	37	604
8 tons rye straw	186	243	138	147	714	46	91	58	45	35	40	51	176	48	590

* One extra 10-gm. portion $NaNO_3$.

† No nitrogen.

‡ Two extra 10-gm. portions $NaNO_3$.

this amount was applied. Certain series also received no nitrogen. These variations are indicated in the tables.

In a number of cases the heavier applications of straw did have a depressing effect on yield, though this did not prove as serious after the experiment had been running for some time as it was the first year.

The yields for three of the crops, soybeans, barley, and oats, for the years 1924 to 1931 are shown in table 1.

In the case of the soybeans grown in 1924 and 1925, it will be noted that organic matter applied at the rate of 8 tons an acre did not have a depressing effect on the yield. It is true that the total yield for the 2 years, with 4 tons of straw is lowest, but this must be attributed to other causes than the depressing effect of the straw since the yields without straw and with 1, 2, and 8 tons to the acre are larger than with the 4 tons.

With the non-legumes—barley and oats—the yields in some cases show a decrease with increase of organic matter, whereas in other cases they run about

constant or even show an increase. For example, the barley grown in 1924 shows a very decided decrease as the amount of organic matter was increased. The yields for the same crop in 1925 tend to show an increase with increased amount of straw rather than a decrease, but this is undoubtedly due to the fact that no straw was applied in 1925. With certain exceptions there is a gradual decrease in the yield for the years 1926 and 1930.

Where no nitrogen was applied for the oats the crop was so poor that the figures have little meaning. When two extra 10-gm. portions of nitrate of soda were applied (cylinders 121–130, 1931), there is no indication of a depressing effect due to the organic matter. With one extra 10-gm. portion of nitrate of soda in 1927, the heavier applications of rye straw had a distinct depressing effect on the yield.

TABLE 2

Yield of dry corn forage—1924–1931

CYLINDER NO. AND DATE / SPECIAL TREATMENT (ACRE BASIS)	151–160 1924	151–160 1925	121–130* 1926	131–140* 1926	151–160† 1926	151–160 1928	141–150‡ 1929	151–160‖ 1929	121–130‖ 1930	131–140‡ 1930	141–150§ 1931	151–160‡ 1931	TOTAL
	gm.	*gm.*	*gm.*	*gm*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
No rye straw	288	288	719	702	342	204	106	180	479	215	752	132	4,407
1 ton rye straw	306	323	734	731	354	205	85	226	597	163	754†	147‡	4,625
2 tons rye straw	240	303	747	778	309	164	76	159	573	148	713	108	4,318
4 tons rye straw	192	310	609	675	294	131	83	149	461	116	591	93	3,704
8 tons rye straw	154	373	642	672	310	103	72	110	629	194	527	114	3,900

* Cylinders 121–140 received nitrogen equivalent to 40 gm. $NaNO_3$ per cyclinder, in two applications.

† 10 gm. nitrate of soda extra.

‡ No nitrogen.

‖ 2 extra 10-gm. portions $NaNO_3$.

§ 3 extra 10-gm. portions $NaNO_3$.

Table 2 shows the yields of dry corn forage grown on the different series of cylinders for the years 1924 to 1931. In some cases no nitrogen was applied; in other cases the normal application was given; and in still other cases extra portions were applied. Where no nitrogen was applied the yield generally decreases as the amount of organic matter applied increases. This also holds true where the single portion of nitrate was applied in 1924 and 1928. It does not hold true for 1925, but no straw was applied for that year, and no doubt the effect of the straw applied in 1924 had largely disappeared. Where the larger amounts of nitrate were applied, the yields did not show the same tendency to decrease with increase of straw as where no nitrogen was applied. Plate 1 shows the effect of the heavy applications of nitrate on the corn.

These figures strengthen the claim that the depressing effect of the organic matter is due to the competition between the growing plant and the micro-

TABLE 3

Percentage of nitrogen—soybeans, barley, and oats—1924–1931

CYLINDER NO. AND DATE / SPECIAL TREATMENT (ACRE BASIS)	SOYBEANS				BARLEY						OATS						AVERAGE
					Grain	Straw							Grain	Straw	Grain	Straw	
	121–130	131–140	121–130	131–140	141–150	141–150	141–150	141–150	141–150*	151–160†	141–150†	151–160*	121–130‡	121–130‡	131–140†	131–140†	
	1924	1924	1925	1925	1924	1924	1925	1926	1930	1930	1927	1927	1931	1931	1931	1931	
No rye straw	2.65	2.28	2.00	2.25	2 04	0 62	1.29	1 64	1 76	1.72	1.03	1.55	2 71	1.30	1.96	1.04	1.74
1 ton rye straw	2 91	2.37	2.05	2.32	1.96	0 52	1 31	1 57	2 12	1 44	0.95	1.45	2 58	1.27	1 91	1.33	1.75
2 tons rye straw	2.74	2.40	2.14	2 28	1.98	0 50	1.29	1 34	1 98	1.35	1.08	1 48	2.62	1 27	2 09	1.20	1.73
4 tons rye straw	2 22	2 32	2 28	2 33	1.82	0 56	1 47	1.32	1.84	1.39	1.14	1 50	2 68	1.19	2.11	1.32	1.72
8 tons rye straw	2.58	2 58	2.11	2 41	1 78	0.68	1.53	1.26	1.70	1.54	1 26	1 54	2 57	1.13	2.45	1.16	1.77

* One extra 10-gm. portion $NaNO_3$.

† No nitrogen.

‡ Two extra 10-gm. portions $NaNO_3$.

TABLE 4

Percentage of nitrogen—corn forage—1924–1931

CYLINDER NO. AND DATE / SPECIAL TREATMENT (ACRE BASIS)	151–160	151–160	121–130*	131–140*	151–160†	151–160	141–150‡	151–160‖	121–130‖	131–140‡	141–150§	151–160‡	AVERAGE
	1924	1925	1926	1926	1926	1928	1929	1929	1930	1930	1931	1931	
No rye straw	0.382	0 353	0.486	0.565	0 592	0.570	0.663	1.116	0.770	0.496	0.447	0.501	0.578
1 ton rye straw	0.387	0.394	0.532	0 541	0 567	0 559	0.702	0 984	0.710	0.520	0.443	0.551	0.574
2 tons rye straw	0.427	0.356	0.532	0 532	0 547	0 678	0 710	0.996	0.596	0.624	0 506	0 551	0.588
4 tons rye straw	0 427	0.381	0.551	0 609	0 564	0.768	0 698	0.984	0.929	0.596	0.517	0 686	0.643
8 tons rye straw	0.527	0.373	0.552	0 651	0 619	0.757	0 704	0 859	0 541	0.801	0.578	0 678	0.637

* Cylinders 121–140 received nitrogen equivalent to 40 gm. $NaNO_3$ per cylinder in two applications.

† 10 gm. nitrate of soda extra.

‡ No nitrogen.

‖ Two extra 10-gm. portions $NaNO_3$

§ Three extra 10-gm. portions $NaNO_3$.

organic population of the soil for the available supply of nitrogen. With a limited amount of nitrogen the plant suffers. With an abundance of nitrate nitrogen the plant gets its needed supply, and the difference in yield between those that receive no straw, or small amounts, and those that receive large amounts, is not great. Table 3 shows the percentage of nitrogen in the dry matter of soybeans, barley, and oats, and table 4 shows the percentage of nitrogen in the corn forage. From the averages in table 3 it is evident that the rye straw has had little or no influence on the percentage of nitrogen in the crop. In some cases the highest percentages are found in crops from cylinders with no rye straw and in other cases in crops from cylinders that received the heavy applications of rye straw.

In the case of the corn forage, table 4, there is in most of the tests, a slight increase in percentage of nitrogen as the amount of rye straw was increased. The increase is slight, however, and may have little meaning.

Calculations of the total nitrogen returned through these crops for the period 1924 to 1931 show the following totals:

	SOYBEANS	BARLEY	OATS	CORN FORAGE
	gm	*gm*	*gm.*	*gm.*
No straw	15 30	6 34	4 83	24 22
1 ton rye straw	17 62	4 82	4 95	25.58
2 tons rye straw	18 26	5 26	4 51	23 76
4 tons rye straw	12 81	4 52	4 27	23 58
8 tons rye straw	17 48	3 92	4 55	23 00

It can scarcely be said that the heavy applications of organic matter influenced the yield of total nitrogen in the soybeans one way or the other. With the barley and corn forage the general tendency is in the direction of a slight decrease with increase of organic matter.

INFLUENCE OF RYE STRAW ON THE NITROGEN AND CARBON CONTENT OF THE SOIL

Samples of soil were collected from these cylinders in 1931 for the purpose of determining the influence of the organic matter on the nitrogen and carbon content of the soil. Table 5 shows the percentages of total nitrogen and carbon and the carbon-nitrogen ratios for the four series with the different organic matter treatments.

Total nitrogen

With slight exception there is a gradual increase in the percentage of nitrogen and carbon with increase of rye straw. An average of the nitrogen percentages for the four series shows a difference of 0.02 per cent between the cylinders without straw and those that receive 8 tons of straw to the acre. This increase seems small, but calculated on the basis of 2,000,000 pounds of soil to the

plowed acre, it would amount to a gain of 400 pounds of nitrogen to the acre during the period 1924 to 1931.

The nitrogen content of this soil, when the work was started in 1924, was 0.0985 per cent and the pH 6.5.

By way of comparison, in field experiments under a 5-year rotation of corn, oats, wheat, and 2 years of timothy, with manure at the rate of 16 tons an acre annually in addition to a complete fertilizer which carries 320 pounds of nitrate of soda an acre, it has taken about 25 years to raise the nitrogen content of the top 6⅔ inches of soil from 0.11 per cent to 0.134 per cent (2).

Total carbon

The total carbon is in reality organic carbon, since carbonates are not present in this soil.

TABLE 5

Total nitrogen, carbon, and carbon-nitrogen ratio in cylinder soils—1931

SPECIAL TREATMENT (ACRE BASIS)	CYLINDERS 121–130			CYLINDERS 131–140			CYLINDERS 141–150			CYLINDERS 151–160		
	Nitrogen	Carbon	Carbon-nitrogen ratio	Nitrogen	Carbon	Carbon-nitrogen ratio	Nitrogen	Carbon	Carbon-nitrogen ratio	Nitrogen	Carbon	Carbon-nitrogen ratio
	per cent	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*	
No rye straw	0.110	1.18	10.7	0.101	1.37	13.6	0.105	1.33	12.7	0.099	1.30	13.1
1 ton rye straw	0.109	1.39	12.8	0.103	1.34	13 0	0.103	1.31	12.7	0.100	1.31	13.1
2 tons rye straw	0 111	1.46	13.2	0 107	1.45	13.6	0.108	1.41	13.1	0.106	1.45	13.7
4 tons rye straw	0.114	1.61	14.1	0 110	1.53	13.9	0.111	1.52	13.7	0.109	1.45	13.3
8 tons rye straw	0.129	1.99	15 4	0.123	1.82	14.8	0.125	1.73	13.8	0.118	1.69	14.3

Table 5 shows that the carbon, like the nitrogen, increases gradually from the cylinders without straw to those that receive 8 tons an acre. The average for all the check cylinders is 1.3 per cent carbon and for those that receive 8 tons of straw 1.81 per cent, an increase of 0.5 per cent. This also seems a small increase, but it serves to indicate how difficult it is to raise the nitrogen and carbon content of soils above the amount present under apparently normal or natural conditions.

Carbon-nitrogen ratio

The figures for the carbon-nitrogen ratio are also shown in table 5. With one exception, the ratio is fairly constant for the first three treatments, that is, with no straw and with 1 and 2 tons to the acre. With one exception, a wider ratio is noted where 4 and 8 tons of the straw were used. This is in agreement with work reported by others. Russell (3, p. 313) in discussing the decomposition of soil organic matter says:

The changes affecting the carbon and those affecting the nitrogen are intimately associated. The nitrogen can appear as nitrate only if it exceeds a certain critical amount relative to the carbon—usually if the ratio C/N is 12 or less. When the proportion of carbon is greater the excess goes off as CO_2 and the nitrogen remains as complex protein: any ammonia or nitrate present is also converted into protein. When, on the other hand, the proportion of nitrogen becomes greater the excess is changed into nitrate which may be taken by plants or otherwise lost from the soil. Whatever the initial composition of the plant substances added, and whatever their form, whether straw, leaves, or animal faeces, the final humus has much the same composition and properties, and its C/N ratio in temperate regions is usually about 10–12; however this be disturbed it always comes back to its normal value.

Organic matter (organic carbon × 1.724)

Organic matter in these soils was calculated from the organic carbon using the factor 1.724. Table 6, cylinders 121–130, shows that the increase in organic matter, due to the rye straw, is greater in this series than for the other series, the difference between the check and the highest rye straw treatment being 1.39

TABLE 6

Organic matter (organic carbon x 1.724) and loss on ignition—cylinder soils, 1931

SPECIAL TREATMENT (ACRE BASIS)	CYLINDERS 121–130		CYLINDERS 131–140		CYLINDERS 141–150		CYLINDERS 151–160		AVERAGE DIFFERENCE BETWEEN ORGANIC MATTER AND VOLATILE MATTER
	Organic matter	Loss on ignition	Organic matter	Loss on ignition	Organic matter	Loss on ignition	Organic matter	Loss on ignition	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
No rye straw	2 04	3.94	2.36	3 95	2 29	4 18	2 24	4 03	1.79
1 ton rye straw.	2 40	4 07	2 30	3 94	2 26	4 18	2 25	4 02	1.75
2 tons rye straw	2 51	4 26	2 50	4 23	2 43	4 15	2 50	4 22	1 73
4 tons rye straw	2.78	4 59	2 64	4 33	2 62	4 34	2 58	4 29	1 73
8 tons rye straw	3 43	5 08	3 14	4 91	2 98	4.91	2 91	4 71	1.79

per cent, whereas the corresponding difference for cylinders 131–140 is 0.78 per cent; for cylinders 141–150, 0.69 per cent; and for cylinders 151–160, 0.67 per cent. The average increase is 0.88 per cent, which, on the basis of the plowed acre, would be equivalent to nearly 18,000 pounds of organic matter.

In the work reported here this increase seems to have proved detrimental rather than beneficial. This detrimental effect is due in part to an excessive amount of the straw and in part to the fact that not sufficient time was allowed to elapse between the time of applying the straw and planting the crop.

In general practice such an increase in organic matter content would mean much in the way of increased water-holding capacity, increased food for micro-organisms, and improved soil tilth.

Loss on ignition

Oven-dried samples of the soil were ignited to determine loss of volatile matter. The results are shown in table 6 along with the organic matter. With

slight exception, the loss increases as the amount of straw applied increases. The average loss for the check cylinders is 4.03 per cent and for those that receive 8 tons of straw to the acre it is 4.9 per cent, a difference of almost 1 per cent in favor of the large application of straw.

It will be noted that the average difference between the percentage of organic matter and the loss on ignition for the different treatments is about 1.75 per cent. This means that for this particular soil the percentage of organic matter could be approximately arrived at by subtracting 1.75 from the percentage of volatile matter. It does not follow, however, that this method of arriving at the organic matter could be used with soils in general.

Soil reaction

The pH of the original soil was 6.5. When the work was started in 1924 pulverized limestone was applied at the rate of 125 gm. for each cylinder (2

TABLE 7

Soil reaction

CYLINDER NUMBER	pH	CYLINDER NUMBER	pH	CYLINDER NUMBER	pH	CYLINDER NUMBER	pH
121	6.48	131	5.86	141	6 44	151	5.95
122	6 14	132	5 75	142	6 30	152	5 93
123	6 63	133	5 79	143	6 48	153	5.86
124	6 50	134	5 69	144	6 30	154	5 74
125	6.10	135	5 74	145	6 74	155	6 00
126	5 84	136	5.77	146	6 58	156	6.15
127	6 63	137	5.85	147	6 88	157	5 95
128	6 58	138	5 84	148	6 62	158	6 10
129	6 41	139	5 97	149	6 25	159	6 07
130	6 51	140	5 95	150	6 58	160	6.16
Average	6.38	Average	5 82	Average	6 52	Average	5.99

tons per acre). Determinations made at intervals during the first 3 years show that the pH ranged from about 6.4 to 7.0. There was no indication of a relation between the pH of the soil and the amount of organic matter applied.

Determinations of pH made July 7, 1932, are shown in table 7.

In this connection it will be remembered that cylinders 121 to 130 and 141 to 150 receive heavy applications of nitrate of soda as a source of nitrogen, whereas cylinders 131 to 140 and 151 to 160 do not receive nitrate of soda or other commercial form of nitrogen. It is interesting in this connection to note that those cylinders which receive the nitrate of soda show, in the majority of cases, a pH between 6.5 and 7.0, whereas those that receive no nitrate of soda, in the majority of cases, show a pH between 5.7 and 6.0. Thus those that receive the nitrate of soda show a pH approximately a half point above those that receive no nitrate of soda. From this it is evident that the nitrate of soda has had a certain corrective effect, in that the reaction has been maintained at near

the neutral point, while those that do not receive nitrate of soda are gradually becoming more acid.

Nitrates

Nitrate determinations were made on samples of the soil at intervals while the crops were growing for the period 1925 to 1930. The results are shown in table 8. The figures indicate that only in a few instances did nitrates accumulate to any extent. Determinations on duplicate cylinders are frequently not

TABLE 8

Nitrates in cylinder soils—1926–1930

(Parts per million)

SPECIAL TREATMENT (ACRE BASIS) / CYLINDER NO. AND DATE OF SAMPLING		CORN		SOYBEANS		OATS		CARROTS	CORN	RYE	AVERAGE
		121–130*	131–140*	121–130	131–140	141–150†	151–160‡	141–150‖	151–160‖	141–150	
		8/25 1926	8/25 1926	7/12 1927	7/12 1927	7/2 1927	6/21 1927	9/6 1928	9/6 1928	5/3 1930	
No rye straw	a	1.00	1.01	1 60	6.80	0.36	0.41	6.16	9.1	2.72	3.24
	b	1.12	0.46	4.32	10 00	0 40	0 41	2.22	8.7	1.51	3.24
1 ton rye straw	a	2 08	0 56	3.00	5.00	0 50	0.81	1.60	8.4	2.39	2.70
	b	0.80	0 48	1.01	6 68	0.37	0 76	2 10	12.0	1.97	2.91
2 tons rye straw	a	0 93	0.72	2.10	2 30	0 33	2 54	1.40	31 0	2.00	4.81
	b	4 00	1 84	2 00	1 90	0 26	2 14	3.20	12.5	2.23	3.34
4 tons rye straw	a	0 84	0.57	1.04	2 68	1 60	0 60	4 40	4 0	2.71	2.05
	b	0.96	0 48	2.00	2 26	0 35	0 80	1.70	7.2	1.35	1.90
8 tons rye straw	a	4 00	0 80	1 32	0 90	0 40	1 50	1 10	5.7	1.32	1.89
	b	1.20	0 42	1 60	1 06	0 54	2 94	5 00	5 3	1.67	2.19

* Cylinders 121–140 received 20 gm. $NaNO_3$ or its equivalent in urea at planting, June 9, and this was repeated on July 29.

† No nitrogen.

‡ One extra 10-gm. portion $NaNO_3$.

‖ One extra 10-gm. portion $NaNO_3$ on carrots and corn, June 28.

in good agreement. This, however, is not surprising, inasmuch as the growth of the crops was not always uniform. The averages for the period, however, indicate higher nitrates with no straw and with 1 and 2 tons of straw than with 4- and 8-ton applications.

SUMMARY

Legume and non-legume crops were grown in Sassafras loam in cylinders without added organic matter and with rye straw, cut fine, varying in amounts from 1 ton to 8 tons an acre. The work was started in 1924 and has been con-

tinued to the present time. Straw has been applied annually according to the plan, with the exception of 1925.

Complete fertilizers were used for most of the work, though in certain series nitrogen was entirely omitted and in others extra quantities of nitrogen in the form of nitrate of soda were used.

With the larger applications of rye straw the yields of non-legume crops were frequently less than the yields on check cylinders. This depressed yield with heavy applications of straw did not occur when legume crops were grown. Likewise there was little or no depression when extra applications of nitrate of soda were made.

In 1925, when no rye straw was used, larger yields were obtained in certain cases from those cylinders which had received the heavy application of straw in 1924 than from the check cylinders, thus showing that the injurious effect of the straw was temporary rather than permanent.

The rye straw had little influence on the percentage of nitrogen in the crop.

Seven years of this treatment did have a distinct influence on the soil. Determinations made on samples of soil collected in 1931 show a gradual increase in total nitrogen, organic carbon, and organic matter as the amount of organic matter applied was increased.

With certain exceptions the carbon-nitrogen ratio was not greatly influenced by the 1- and 2- ton applications of straw, but with 4 and 8 tons to the acre a wider carbon-nitrogen ratio was found.

The heavy applications of nitrate of soda have tended to keep the soil reaction near the neutral point. Where no nitrate was used the soil is gradually becoming more acid.

Nitrate determinations made at intervals during the period indicate that an abundance of organic matter may depress nitrate formation.

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PLATE 1

Effect of Heavy Applications of Nitrate on Corn

The row of corn on the left received an extra heavy application of nitrate; the row on the right received no nitrate. Cylinders in the immediate foreground (both rows) received the rye straw at the rate of 8 tons to the acre.

THE DEGREE OF HUMIFICATION IN MANURES MEASURED BY THE USE OF HYDROGEN PEROXIDE

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It has been shown by Robinson and Jones (4) that humified organic matter can be distinguished from non-humified by the use of 6 per cent hydrogen peroxide. An account is given in the present paper of the use of this reagent in determining the degree of humification in manures. Different workers have employed different concentrations of the reagent. McLean (2) made a thorough investigation with the reagent in various concentrations in estimating the organic matter in soils. Jones (1) used 6 per cent hydrogen peroxide in determining the degree of humification in farmyard manure. Humified or decomposed organic matter appears to be oxidized or rendered soluble by the reagent while the structural or undecomposed materials remain unaffected. It has been assumed by Jones that such a distinction exists in the case of farmyard manure between the decomposed material and the unaltered fiber of the feces and litter. In the present investigation the manures were obtained by artificial methods and no such assumption is made.

Richardson (3) holds that if a reagent is to be used for effecting a separation between humified and non-humified matter it should have a minimum of action on the unhumified matter. He observes that with most materials the greater part of the effect of hydrogen peroxide is simply a solvent action. This means in effect that[2] water alone can reproduce most of the loss by its solvent action. To test the validity of Richardson's statement simliar samples of manure were treated simultaneously under identical conditions with (*a*) water, (*b*) hydrogen peroxide. The time required for the reaction with H_2O_2 to be completed, i.e. when frothing ceased, was first found. The samples were then treated with water for the same length of time with the same volume as peroxide. This gave a comparison between the solvent action of both H_2O_2 and water and also made evident the further action of the reagent over and above that of water. In each of the representative cases the loss of ash and the loss of organic matter due to extractions with both reagents were also compared.

[1] The author is indebted to Sir John Russell, director of the Rothamsted Experimental Station for placing at his disposal the facilities of the station. His thanks are due to Mr. E. H. Richards, head of the fermentation department and to Dr. S. H. Jenkins for their assistance and advice.

[2] With many materials.

In view of the differences of opinion held regarding the use of 3 per cent or 6 per cent hydrogen peroxide, both strengths were tried with chaffed oat straw and with the same straw rotted in the presence of ammonium carbonate. No appreciable difference was found between the results obtained in both cases. It was therefore decided to use 3 per cent hydrogen peroxide instead of 6 per cent in all the quantitative experiments.

EXPERIMENTAL

Oat straw was rotted under aerobic conditions at 35°C. with initial moisture contents of 60, 70, 80, and 90 per cent for each nitrogenous substance. Each bottle, containing 20 gm. of the air-dry straw, was supplied with 0.2 gm. of nitrogen in the form of inorganic salts such as ammonium carbonate, ammonium sulfate, and sodium nitrate, and organic substances such as peptone, casein, and urea. The bottles were incubated for one month and the contents mixed at frequent intervals. Whenever necessary, moisture was added but the manures were never allowed to become water-logged. At the end of 1 month the bottles were weighed and the moisture contents together with other analytical figures were determined. The loss of dry matter on rotting was then calculated and the extent of the decomposition found.

The total percentage of organic matter was determined by finding the "loss on ignition" on 1 gm. of the well-chopped manure dried at 100°C. The quantity of the manure available for analysis being small, about 1-gm. samples of the same dry manure were used for treatment with hydrogen peroxide. The material was gradually heated to boiling with 100 cc. of 3 per cent peroxide with stirring in beakers of 600 cc. capacity. For this reaction, 100 cc. of the reagent was found to be enough. When the frothing had ceased, which indicated that the reaction was complete, the contents of the beaker were made up to about 250 cc. with hot distilled water and boiled for 20 minutes. The contents were then filtered hot, first by decantation of the supernatant layer of the solution on a tared fluted filter paper. The residue was thoroughly washed with hot water till the filtrate was colorless, when it was finally tranferred to the filter and dried at 100°C. It was then cooled in a desiccator and rapidly weighed, as the material absorbs moisture. The residue and filter paper were ashed together and the ash of the filter paper was deducted. The weight of the organic matter initially present being known, the amount lost by oxidation or by solvent action or from both causes can be obtained by difference. The total loss is obtained by subtracting the residue after H_2O_2 treatment from the weight of the dry manure before its treatment with the reagent. This total loss is always found to be more than the loss in organic matter, as it is likely that some of the inorganic material will dissolve out. The loss of organic matter on 100 gm. of the original material gives the degree of humification.

To test whether the loss due to the peroxide treatment was due to oxidation or to solvent action or to both causes in each of the representative cases, the

hydrogen peroxide extract was evaporated to dryness in weighed silica dishes on a water bath and then dried in a desiccator. The ash in the extract was obtained by ignition. The residue in the peroxide extract minus the ash of this extract gives the organic matter present in the extract. The total loss minus the peroxide residue gives the matter oxidized by the action of peroxide.

For comparing the action of water and peroxide, about 5 gm. of the wet samples were extracted with the same volume of water as peroxide under similar conditions. In the case of water extract direct filtration is slow and hence it was first filtered through a plug of cotton wool, and then in a Buchner funnel on a water suction pump. The residue was well washed with hot water, The filtrate was evaporated to dryness on a water bath, weighed, and the total extract was determined by igniting the dried residue.

To determine how the method worked with manures, three different types, which had been rotted under known conditions, were treated first. One

TABLE 1

Results expressed on 100 gm. of dry manure

	MOISTURE CONTENT	TOTAL O. M *	O M AFTER H_2O_2 TREATMENT	LOSS OF O. M.	TOTAL LOSS	DEGREE OF HUMIFICATION
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*
1	82 2	71.9	23 9	48 0	59 0	66 7
2	83 0	55 2	24 7	30 5	36 8	55 2
3	76 0	56.5	44 2	12 3	22 7	44 2

* O. M. = organic matter in this and following tables.

sample was a highly humified farmyard manure. The second was an artificial farmyard manure made from wheat and bean straw rotted with calcium cyanamide as the added supply of nitrogen, and was fairly well humified. The third was wheat straw alone also rotted with cyanamide. It did not appear to be well humified. The results of the treatment of the three manures with peroxide are given in table 1 and seem to agree with the appearance of the samples. The duplicate determinations agreed closely. The difference between the action of 3 per cent and 6 per cent hydrogen peroxide on undecomposed and decomposed straws is not appreciable, as can be seen from table 2.

The loss of organic matter in the case of undecomposed straw is about 20 per cent in both the treatments and in the case of both artificial manures the average loss amounts to about 30 per cent. As there was no difference in the amount of extractable matter obtained with the two strengths of peroxide, the 3 per cent solution was chosen for further experiments. The only noticeable difference was in the colors of the filtrates and the residues. The color in the case of 6 per cent was paler than in the case of 3 per cent. This color difference can be attributed to the bleaching action of the peroxide.

Table 3 gives the results obtained with 3 per cent peroxide on the various artificial manures.

From a study of the figures in table 3 it can be seen that in general the loss of organic matter after peroxide treatment increases with the increase in the initial moisture content, and the percentage loss of dry matter during incubation. There is a corresponding increase in the apparent degree of humification with the increase in the loss of organic matter. The greater the moisture content of the manure and the greater the degree of rotting as measured by loss in dry weight, the greater is the amount of organic matter removed by the peroxide treatment. It is evident from the figures indicating the total loss

TABLE 2

Straw alone

TREATMENT	TOTAL O M.	O M. AFTER TREATMENT	LOSS OF O. M	TOTAL LOSS	DEGREE OF HUMIFICATION	WATER EXTRACT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
3 per cent H_2O_2	93 56	73 90	19 66	21.85	21 02	12 1
6 per cent H_2O_2	93 56	72 89	20 67	23 20	22 10	12 1

Artificial manure made from straw fermented with ammonium carbonate

MOISTURE CONTENT	LOSS OF DRY MATTER	TREATMENT	TOTAL O. M.	O M AFTER TREATMENT	LOSS OF O. M.	TOTAL LOSS	DEGREE OF HUMIFICATION	WATER EXTRACT
per cent	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
80 3	36 5	3 per cent H_2O_2	90.12	62.94	27.18	31 9	30 16	21.14
85 0	45 8	3 per cent H_2O_2	87 26	55 64	31 62	38 2	36.00	21.23
86 0	39 2	3 per cent H_2O_2	88 90	50 60	38.30	43.41	43 08	22.23
88.6	44.4	3 per cent H_2O_2	87.50	62.90	24.60	34 52	28 12	29 30
80 3	36 5	6 per cent H_2O_2	90.12	64.73	25.39	30.01	28.17	21 14
85.0	45.8	6 per cent H_2O_2	87.26	55.11	32.15	39 22	36 85	21.23
86.0	39 2	6 per cent H_2O_2	88.90	72 30	. . . .	. . . . .	. .	22 23
88 6	44 4	6 per cent H_2O_2	87.50	54 60	32 90	39.60	37 60	29 30

due to peroxide treatment and to the water extract that the loss is greater with peroxide than with water extraction. This point is also apparent in table 4 where the residue on evaporation of peroxide extract and its ash content are compared with the residue in the water extract and its ash content. In these estimations all the samples of one particular artificial manure were mixed up as evenly as possible and the extractions were then carried out as given in the foregoing. From a consideration of the figures in columns 4 and 8 the residues in water extract is always greater than in peroxide, whereas the total loss in column 3 is always greater than that in column 8. Total loss minus the residue in peroxide extract gives the matter oxidized by the reagent. The residue in the peroxide minus the ash (column 6) gives the organic matter in the peroxide extract which remains quite unaffected by the reagent, i.e., not

oxidized but only dissolved. The figure of 8.8, which is the organic matter in the resistant form in the peroxide extracts, seems to be of some significance. It is constant in five cases out of seven. This resistant form of organic matter may be compared with McLean's resistant form of nitrogen in the soil organic matter. In the first two cases when the figures are higher than 8.8 the filtrate was turbid. This turbidity may be due to the presence of a special type of

TABLE 3

MOISTURE CONTENT	LOSS OF DRY MATTER	TOTAL O M.	O M AFTER TREATMENT	LOSS OF O M.	TOTAL LOSS	DEGREE OF HUMIFICATION	WATER EXTRACT
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Straw rotted with $(NH_4)_2SO_4$							
76 54	30.1	88 98	72 70	16 28	22 90	18 30	17 94
80.70	32 5	88 45	63 8	14.65	30 68	16 53	20 50
83 30	31 6	88 52	69 5	19 02	30.51	21 50	20.84
85.25	30 7	88 86	65 21	23 65	29 75	26 61	19 84
*Straw rotted with $NaNO_3$**							
84.80	52 8	78 68	35 85	42 83	57 94	54 44	44.36
86 70	52 3	77 90	33 15	44 75	62 70	57 45	62 10
Straw rotted with H_2O							
81.75	30 0	89.42	73 25	16 17	21 27	18 08	12 93
Straw rotted with peptone							
83 30	39 5	88 38	61 05	27 33	32 53	30 92	21 53
84 42	44 1	87 32	58 84	28 48	34 99	32 61	26 90
Straw rotted with casein							
82 9	41 8	87 60	54 10	33 50	39 33	38 24	27 20
83 6	43 0	85 70	54 70	31.00	38 33	36 20	28 90
Straw rotted with urea							
80 0	32 5	86 95	68 40	18 55	26 70	21 34	19 30
80 5	34 0	88 74	62 40	26 34	32 00	29 46	25 30

* This sample rotted for 45 days; other samples for 30 days only.

organic matter containing a large amount of carbohydrate material or the residues from the fungi which were seen to grow very profusely in both cases. The extraordinarily high figures in the case of sodium nitrate are no doubt due to the high degree of rotting, because the straw was rotted for over 1½ months as compared with 1 month in all the other cases. A comparison of the sodium nitrate manure with the farmyard manure, which was also well rotted, shows that the amount of organic matter after peroxide treatment and matter oxidized in each case respectively are of the same order. The quantity of

organic matter in the water extract is decidedly higher than that in the peroxide extract. Hydrogen peroxide also extracts inorganic material but the ash con-

TABLE 4

Comparison of residue on evaporation of peroxide extract and its ash content with residue in water extract and its ash content

SUBSTANCE USED FOR ROTTING THE STRAW	INITIAL O. M.	O. M. AFTER H_2O_2 TREATMENT	TOTAL LOSS	RESIDUE IN H_2O_2 EXTRACT	MATTER OXIDIZED	ASH IN H_2O_2 EXTRACT	O. M. IN H_2O_2 EXTRACT	RESIDUE IN WATER EXTRACT	ASH IN WATER EXTRACT	O. M. IN WATER EXTRACT
	1	2	3	4	5	6	7	8	9	10
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
$(NH_4)_2CO_3$	88 5	59 85	35 86	20 27	15.59	4.84	15 43	31.0	20.2	10.8
$NaNO_3$	78.3	27.30	68 80	27 40	41 40	14 60	12 80	57.8	25.0	32.8
$(NH_4)_2SO_4$	88 6	66 42	32.19	14 50	17 69	5 89	8 61	21 0	8 8	12.2
Urea	87 7	62.71	32 04	12 87	19.17	4.00	8 87	29 6	7 6	22 0
Peptone	87 8	53.20	40.30	16.35	24 00	7 80	8 55	31 8	9.2	22 6
Casein	86.9	56 30	36 90	15 30	21.60	6 47	8 83	27.2	7.1	20.1
Farmyard manure*	71.9	23 11	61 28	20 28	41 00	11 43	8 85	31 08	11.08	20 0

* Farmyard manure used alone.

TABLE 5

Extractive properties of peroxide and water

	LOSS ON 100 GM. OF THE DRY MANURE AFTER						NET LOSS DUE TO PEROXIDE TREATMENT		
	A. Water extraction			B. Peroxide extraction			B − A		A − B
	Total loss	Organic matter loss	Ash loss	Total loss	O. M. loss	Ash loss	Total loss	O. M. loss	Ash loss
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Straw	12.1	9 1	3.0	21.9	19.7	2.2	9.8	10 6	0.8
Straw rotted with $(NH_4)_2CO_3$	31.0	10 8	20.2	36.5	30 0	6.5	5 5	19.2	13.7
Straw rotted with $(NH_4)_2SO_4$	21.0	12.2	8 8	28.0	18.0	10.0	7.0	5.8	....
Straw rotted with $NaNO_3$	57.8	32.8	25 0	61.0	43 5	17.5	3.3	10.7	7.5
Straw rotted with H_2O	13.0	7.1	4.9	21.3	16.2	5.1	8 3	9 1	....
Straw rotted with peptone	31.8	22.6	9 2	33.5	27.7	5.8	1 7	5 1	3.4
Straw rotted with casein	27.2	20.1	7.1	38.5	32.2	6.3	11.3	12.1	0.8
Straw rotted with urea	29.6	22 0	7.6	29.3	22.4	6.9	....	0.4	0.7
Farmyard manure	31.08	20.0	11.08	59.0	40.0	19.0	28.0	20.0	....

tent of the water extract is, in general, higher than that of the peroxide. The extractive properties of peroxide and water are further compared in table 5. Table 6 gives the action of peroxide on the manures extracted with alcohol.

It will be seen, in comparing the average figures for loss in organic matter, total loss, and degree of humification in the various columns in tables 1, 2, and 3 with the corresponding ones in table 6, that those in tables 1, 2, and 3 are in most cases lowered by alcoholic extraction. Alcohol removed some of the organic and inorganic matter from the manures.

The degree of humification thus appears to be a useful measure of the decomposition undergone by any one kind of plant material under different treatments. It must not be regarded as an infallible guide to the value of organic manures in general; still less as an index of the biological availability of the end products.

TABLE 6

Action of peroxide on manures extracted with alcohol

	TOTAL O. M.	O. M. AFTER H_2O_2 TREATMENT	LOSS IN O. M.	TOTAL LOSS	DEGREE OF HUMIFICATION
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Straw	95.95	80 12	15 83	16 81	16.50
Straw rotted with $(NH_4)_2CO_3$	90.32	69 93	24 39	24.48	27 00
Straw rotted with $(NH_4)_2SO_4$	87 10	72 75	14 35	22 48	16 48
Straw rotted with $NaNO_3$	81 20	51 90	21 30	34 58	26 23
Straw rotted with peptone	89 45	49 42	40 03	44 04	44 76
Straw rotted with casein	87 95	65 73	22 22	27 17	25.27
Straw rotted with urea	92 00	62 43	29 57	33 03	32 14
Straw rotted with H_2O	92 99	93 20	.	20 85	. . .
Farmyard manure	37 00	28 80	8 20	22 10	52 20

SUMMARY

The action of 3 per cent and 6 per cent hydrogen peroxide on artificial manures has been studied and it has been found that there is not much difference in the action of the two concentrations of this reagent. Peroxide attacks undecomposed straw to a certain extent.

Artificial manures can be arranged in order of rotting according to the figure for apparent degree of humification derived from loss in weight due to extraction with H_2O_2. Higher initial moisture content in a series of artificial manures results in a greater degree of humification.

In comparing the action of peroxide and water on straw and manures of different origin it has been found that (*a*) the effect of peroxide is more than a solvent action such as is the case with water, (*b*) the water extract contains more organic matter than the peroxide extract, (*c*) water appears to extract more inorganic substance than peroxide, (*d*) the amount of organic matter in the peroxide treatments is generally constant whereas that in the water shows greater variation.

In a series of artificial manures made from straw and various sources of nitrogen the degree of humification could be arranged in the following order,

proceeding from the highest to the lowest: {$NaNO_3$ and $(NH_4)_2CO_3$}, casein, peptone, urea, $(NH_4)_2SO_4$.

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SOME OBSERVATIONS ON BASE EXCHANGE IN ORGANIC MATERIALS[1]

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Although the study of the base exchange phenomenon in mineral soils has been carried to a high degree of attainment, it is only recently that any material progress in the study of base exchange in organic materials has been reported.

Observations and limited studies of this problem were made by several early workers. Rautenberg (14), in 1862, observed base exchange in forest humus; Heiden (5), in peat and various peat preparations; and Eichorn (3), in peat and calcium humate. König (7), in 1882, published a paper giving a review of the work done up to that time, and also reported a long series of experiments which he performed determining the absorption of acids, salts, and bases. He concluded that the absorption process was chemical in nature.

Van Bemmelen (1), continuing his studies of mineral colloids to include humic substances, showed that they form adsorption complexes with acids, salts, and especially bases. These are not, he pointed out, ordinary chemical compounds; they are adsorption compounds of various proportions, depending upon such factors as concentration, temperature, and the nature of the gels and solutions concerned.

An observation which is believed to be of particular significance in the light of the results given later in this paper was reported by Witz (25) in 1882–83. While examining cotton fabrics which had been accidentally overbleached, he observed that the edges of the resulting holes had a strong affinity for basic dyes. The product could be reproduced by the use of various oxidizing agents and was found to contain less carbon and more oxygen than normal cellulose and was given the name "oxycellulose."

It appears, however, that until quite recently there had been no systematic study of this whole problem. The rapid advance in fundamental knowledge of the chemical structure of plants and its relation to the resulting decomposition products has stimulated work along all lines of soil organic matter. Odén (12) has made an extensive study of humus and humus complexes, particularly from the physico-chemical standpoint. He has regarded the humic substances as true acids. A comprehensive review of this subject of soil organic matter, together with an extensive bibliography, has been published by Waksman (18).

Burgess and McGeorge (2) began working on base exchange in soil organic material while studying soil zeolites. Since then McGeorge (8, 9) has published further results of his investigations. He has pointed out the relatively high replacement capacity of the ligno-humate fraction; the formation of synthetic humus, having replacement power, from xylan, sucrose,

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[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology. The data are those presented in a thesis submitted to Rutgers University in partial fulfilment of the requirements for the degree of doctor of philosophy, 1930.

[2] The writer wishes to express his appreciation to Dr. Sante Mattson, of this laboratory, for suggesting the problem and for his many helpful suggestions and criticisms offered during the progress of the investigation.

and cellulose; the probable lack of relationship between the nitrogenous or protein fractions and base exchange; the replacement capacity of raw plant material (alfalfa); and the rapid increase in replacement capacity with decomposition. He has concluded that the lignin or ligno-humate material is most active in base exchange, but qualifies this with the necessary statement that because of the variability in the chemical composition of lignin, and the various forms of lignin-carbohydrate linkages, the replacement capacity of materials of plant origin is not a direct function of the lignin content. He believes "the exchange capacity, both in lignin and ligno-humate is a function of the phenolic hydroxyl groups in the molecule.—As the carbohydrates are removed by chemical and biological reactions,—the phenolic hydroxyl groups are rendered free to act and the exchange capacity of the complex increases accordingly."

Mitchell (11) carrying on somewhat similar investigations also concludes that lignin, or a derivative of very similar nature, is the constituent responsible for the base exchange reactions of the ligno-humate fraction of the soil organic matter.

METHODS

In determining the base exchange in the following experiments, the material was treated with 400 cc. of 0.2 *N* barium acetate solution, leached twice with *N* barium chloride solution, washed with distilled water until no test for chlorides was observed, and then leached with 400 cc. of *N* ammonium chloride solution, and the replaced barium determined in the filtrate. The samples varied from 1 to 5 gm., dry basis. The moisture and ash contents were determined on separate samples.

The chemical treatments used were as follows:

Hydrolysis by treatment with 80 per cent sulfuric acid, 10 cc. per 1 gm. of material, in the cold for 2 hours. The acid then diluted with 15 volumes of distilled water and boiled for 3 hours under a reflux condenser [see Fred and Waksman (4)]. This method will be referred to as "hydrolysis treatment."

Treatment with 80 per cent sulfuric acid, 10 cc. per 1 gm. of material, in the cold for 2 hours. The acid then diluted with 16 cc. of distilled water per 10 cc. of 80 per cent acid used and boiled under a reflux condenser for 3 hours. This method will be referred to as "strong acid treatment."

Treatment with sulfuric acid, specific gravity 1.84, 100 cc. per 10 gm. of material. No heat is applied although some heat may be generated during the reaction. At the end of 3 days the mixture is poured into 600 to 1,000 cc. of distilled water [see Tschumanow (16)]. This will be referred to as "concentrated sulfuric acid treatment."

Treatment with 10 per cent sodium hydroxide solution, 10 cc. per 1 gm. of dry material, under pressure at 120° to 130° for 2 hours. Three volumes of hot distilled water are added and the residue filtered off. The dark colored liquor is treated while hot with hydrochloric acid in slight excess to precipitate the dispersed fraction [see Powell and Whittaker (13)]. This procedure will be referred to as "extracted with 10 per cent sodium hydroxide." The residue will be known as "non-dispersible residue," and the hydrochloric acid precipitate as the "dispersed fraction."

Oxidation by chlorine to give oxycellulose. Ten grams of material is added to a boiling solution of 50 gm. of potassium chlorate in 1,000 cc. of water, and 42.5 cc. of 35 per cent hydrochloric acid is gradually added. The mixture is held at the boiling point for 1 hour [see Vignon (17)]. This will be referred to as "oxidation by chlorine."

Oxidation by potassium permanganate. Thirty grams of material is treated with 600 cc. of 6.5 per cent sodium hydroxide solution and shaken until a pulp is formed, which is then thinned with water, made acid with sulfuric acid, filtered off by suction, and washed with

water. The moist pulp is suspended in 600 cc. of 7.5 per cent sulfuric acid solution and rapidly stirred mechanically. Twelve grams of potassium permanganate dissolved in 7.5 per cent sulfuric acid is slowly added by means of a dropping funnel, 1 to 2 hours being taken for the addition. The solution becomes decolorized after several hours. The brown precipitate is then filtered off, suspended in dilute sulfuric acid, decolorized by hydrogen peroxide, and washed with water until free of acid and manganese sulfate. It is dried at a temperature not exceeding 40°C. [see Knecht and Thompson (6)]. This will be referred to as "oxidation by permanganate.".

Ether extraction, carried out in the usual way by means of Soxhlet extractors.

THE BASE EXCHANGE CAPACITY OF ORGANIC MATERIALS

In order to formulate a classification of a group of various organic materials from the standpoint of base replacement, a series of determinations was made on these materials. All of the substances studied were first ground to pass a 2-mm. sieve or finer.

The "Natural Humus" and "Hyper-Humus," both of these being trade names, are black peats from northwestern New Jersey dug for agricultural use and partially air dried. The German peat moss was of the usual quality imported for stable use. The leaf mold was obtained in southern New Jersey and was a forest ground covering. The sphagnum moss was growing when gathered about 6 months prior to the time that it was used in this experiment and had been thoroughly air dried. The Michigan peat was largely wood in various stages of decomposition. The brown rot was a sponge-like mass obtained from the base of a large living black oak tree near Princeton, New Jersey. The oak leaves were dead, but still attached to the twigs of the tree when gathered. The Barr Bog peat was from Michigan and consisted of only partially decomposed swamp growth. The bagasse was a by-product from the manufacture of "Cellotex," a commercial insulating wall board, and was artificially dehydrated. The shavings were of hardwood and of the type commonly employed as litter in stables.

The results of the base exchange determinations on these various materials are given in table 1.

Table 1 shows that the degree of decomposition and the base exchange are more or less correlated, the materials such as the peats having a high base exchange and the fresh materials such as straw and wood shavings having little or none. However, this generalization does not hold in all cases. The sphagnum moss and oak leaves although entirely undecomposed, showed a relatively high base exchange.

Effect of decomposition on the base exchange capacity of organic materials

In order to determine what magnitude the effect of decomposition might assume, and the rapidity with which it would become effective, quantities of ground straw and dehydrated cow manure (with peat moss litter), inoculated with a few cubic centimeters of soil extract and kept moist, were allowed to decompose under aerobic conditions. The results of the base exchange determinations are presented in table 2.

The gradual increase in the base exchange of the straw as decomposition

progressed is particularly interesting, since so little base exchange is apparent in the original material. According to Waksman and Tenney (22) the aerobic decomposition of straw is accompanied by a gradual disappearance of the cellulose and hemi-celluloses and an accumulation of the lignin. Accumulated

TABLE 1

Base exchange in organic materials

MATERIAL	BASE EXCHANGE PER GRAM DRY WEIGHT	ASH (DRY BASIS)
	m.e.	*per cent*
Peats and Humus:		
"Natural Humus"	1.73	25.48
German peat moss	1.43	1.24
"Hyper-Humus"	1.39	11.58
Leaf mold	1.18	21.68
Michigan peat	1 07	3.27
Bar bog peat	0 66	8 47
Plant materials:		
Sphagnum moss	1 12	5 72
Oak leaves	0.95	3 31
Bagasse	0.13	3.30
Rye straw	0.12	4.97
Wood shavings	0 00	0 70
Miscellaneous:		
Brown rot	1 07	2.83
Cellulose (filter paper)	0 00	0.18

TABLE 2

Base exchange in decomposing organic materials

TIME	BASE EXCHANGE PER GRAM DRY WEIGHT	
	Straw	Manure
	m.e.	*m e.*
Beginning	0.12	0.73
3 weeks	0 22	1.03
8 weeks	0.30	1.09
14 weeks	0 33	1.11
24 weeks	0.38	1.45
36 weeks	0.59	1 67

lignin, together with synthesized microbial cell substance, is believed by Waksman (18) to make up a large part of the natural soil humus.

Base exchange in organic materials as influenced by chemical treatments

It has been shown that natural decomposition processes intensify the base exchange in organic materials. These processes, however, are slow, and so

many different processes are at work simultaneously that even by analysis of the end products it is impossible to say just what compounds or complexes are present at any given time. In order to study this question under somewhat better controlled conditions, a series of chemical treatments was selected and various organic materials, both natural plant materials and pure substances,

TABLE 3

Base exchange in organic materials as influenced by chemical treatments

TREATMENT	STRAW	CELLULOSE	WOOD SHAVINGS	SPHAGNUM MOSS	HUMUS	"NATURAL HUMUS"	BROWN ROT
	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.
No treatment	0 12	0 00	0 00	1.12	2.65	1.73	1.07
Ether extraction	0 13		0 00				
Hydrolysis treatment	0 10		0 20	1 40	1.96	1.65	1.06
Dry heat, 300°C	0 27						
Strong acid treatment	1.02		0.52	1.62	1.75	1.46	1.09
Extracted with 10 per cent sodium hydroxide:							
Non-dispersible residue	0 00		0 33	1 37		2.39	
*Hydrolysis treatment	0 00						
Strong acid treatment	0 88						
Oxidized by chlorine	0 24						
Dispersed fraction	1 10		0 48	1 42		2.65	1.68
Hydrolysis treatment	0 47						
Strong acid treatment	0 36					2 43	
Oxidation by chlorine	0 59	0 18			1 91		1.31
Extracted with 10 per cent sodium hydroxide:							
Non-dispersible residue	0.39						
Dispersed fraction	1.14						
Oxidation by permanganate		0 10					
Extracted with 10 per cent sodium hydroxide:							
Non-dispersible residue		0.16					
Dispersed fraction		0.33					
Reprecipitated from Schweitzer's reagent		0 00					
Concentrated sulfuric acid treatment		1 30					
Extracted with 10 per cent sodium hydroxide:							
Non-dispersible residue		3.71					

* The sub-treatments were applied to the residues of the respective original treatments.

were subjected to them. These treatments have been described under *Methods*. All of the results, recorded in table 3, are given as base exchange capacity, milli-equivalents per gram of dry material *remaining after the treatment.*

The materials used have all been described with the exception of the cellulose, which was Whatman no. 2 filter paper containing 0.0029 gm. ash per 15-cm.

sheet, equivalent to approximately 0.18 per cent ash; and the humus, which was prepared by Mattson, who has given the method of preparation elsewhere (10).

DISCUSSION

If the classification of plant material constitutents as arranged by Waksman and Tenney (21) are accepted as a basis for consideration of the data presented, there are the following groups:

Ether-soluble substances: oils, fats, and waxes. It is probable that when aqueous salt solutions are used, the base exchange cannot be associated with this group in general. Furthermore, these substances are comparatively resistant to decomposition by microörganisms (19), and table 2 shows an increase in base exchange in straw almost as soon as decomposition begins. It is possible, however, that there may be some relationship of this group under certain conditions.

Water-soluble substances, including the readily dispersible colloidal substances: sugars, tannins, pectins, starches, amino acids, and other organic acids. The true water-soluble substances obviously cannot be concerned. The others have not been studied, but as McGeorge (9) points out there is a possibility that the pectins possess base exchange properties. Preliminary experiments in this laboratory also indicate the possibility of base exchange in alumino-tannate complexes.

Hemicelluloses. These are principally lyophyllic colloids, present in most plant material, either fresh or decomposed. Tenney and Waksman (15) give the hemicellulose content of rye straw as 21 per cent and of oak leaves 13 per cent, and the hemicellulose content of peat has been shown to vary from 5 to 17 per cent (20). No intensive study seems to have been made of this group of substances from the standpoint of base exchange, but Mitchell (11) concludes from his work that they do enter into the reaction.

Cellulose. No base exchange was observed in untreated cellulose or reprecipitated cellulose (table 3), nor has any mention of such observations by other investigators been seen. McGeorge (9) has reported a base exchange capacity of 0.4 m. e. per gram of cellulose after treatment with 10 per cent hydrochloric acid on the steam bath, the resulting product being a dark brown mass. A considerably greater base exchange capacity was found in this laboratory when filter paper was treated with concentrated sulfuric acid, as shown in table 3. The resulting product was black, and became hard and lustrous upon drying. According to Tschumanow (16) the composition is 62 per cent carbon, 5 per cent hydrogen, and 33 per cent oxygen, corresponding to the formula $(C_2H_2O)_n$ and approaching "ulmic acid" in composition. Here then is a material derived from pure cellulose having much the appearance of a humic substance, being colloidal in nature, and having a relatively high base exchange capacity. A fraction may be dispersed in strong sodium hydroxide solution under pressure and reprecipitated by hydrochloric acid, the method for extracting lignin. The non-dispersible residue exhibits a very high base exchange capacity.

Cellulose oxidized by chlorine or permanganate also has been shown to possess a base replacement capacity. Since oxidation is probably the most common of the natural chemical processes, it would seem reasonable to suppose that, under conditions unfavorable to the complete breaking down of the cellulose, oxycellulose might result. Winogradsky (23, 24) has mentioned isolating several forms of cellulose decomposing bacteria which gave oxygen-saturated products resembling soil humus in being colloidal, nitrogenous, resistant to further bacterial attack, and soluble in dilute alkalies. He believed that "they remain for a long time components of the organic colloids" of the soil. These products were quite possibly mixtures of oxycellulose and bacterial cell substance.

Lignin. Undoubtedly this substance is largely concerned in base exchange in natural

organic materials. McGeorge (9) and Mitchell (11) have both concluded that this is the most active of all of the soil organic matter fractions. The results given here also lead to that general conclusion. However, it should be borne in mind that lignin varies in composition in different species of plants and even in different parts of the same plant. Furthermore, the methods of isolation involve such drastic treatments that it is impossible to obtain lignin unchanged from its natural state. This is true not only of its chemical structure but also of its base exchange capacity, as is shown by the difference between "alkali' lignin and "acid" lignin obtained from straw. However, it seems logical to assume that lignin is largely responsible for base exchange in natural organic materials.

These few observations recorded in the accompanying tables, while not extensive enough to be used as any definite basis for hypotheses, do indicate, it is felt, the trend which further investigations should follow to solve this problem. It is apparent that no one substance is wholly responsible for base exchange in organic materials. Furthermore, the chemical treatments used to isolate the various plant material fractions are so drastic as to preclude the assumption that those isolated are identical in structure with those in the plant before isolation. Probably complex combinations of two or more substances, as well as intermediate degradation products, enter into the reaction. It is felt that no real progress can be looked for by simply making a large number of base exchange determinations on these innumerable substances.

Considering again the results obtained with cellulose (table 3), for example, it is shown that untreated cellulose, as represented by finely divided filter paper, has no base exchange capacity. Reprecipitated cellulose has none. Oxidized cellulose, obtained by two different methods, shows some; and cellulose treated with strong sulfuric acid and then strong alkali has a base exchange capacity greater than natural or purified humus. Pure cellulose is the only substance making up this exchange complex, but it has been so acted upon by the reagents used that it is no longer recognizable, and in truth is no longer cellulose. In the turmoil its structure has been rearranged from one having no base exchange capacity to one having a very great one.

The answer must be sought further than simply as to the identity of the substances making up any particular material. The chemical structure must be studied. It is strongly believed that free valences are the determining factor. Free carboxyl or hydroxyl groups or free hydrogens should be sought as determining the base exchange capacity. No doubt other groups may also be involved. Before trying to determine in each of innumerable individual cases with what particular substance the base exchange property is associated, it would appear more logical, by using materials of known chemical structure, to determine to what radicals this phenomenon is attributable. Then it would be but a step to postulate the occurrence of base exchange under certain definite conditions.

Natural organic materials, and particularly soil organic matter, are in a dynamic state, building up on one side and tearing down on the other. Under these unstable conditions, it must be concluded that base exchange capacity is determined by the chemical structure existent under the conditions of and at

the moment of the determination, and is not simply a property of some particular substance present.

SUMMARY

Various natural organic materials were shown to have considerable base exchange capacities.

Decomposition by natural processes increased the base exchange in straw and manure.

The base exchange of several organic materials was markedly altered by certain chemical treatments.

It is concluded that under the present methods of drastic chemical treatments required for the isolation of the various fractions of organic materials, the property of base exchange should be assigned to certain groups such as hydroxyl or carboxyl, rather than to specific chemical substances.

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LYSIMETER STUDIES: II. THE MOVEMENT AND TRANSLOCATION OF SOIL CONSTITUENTS IN THE SOIL PROFILE

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Notwithstanding the vast volume of work reported on the physical, chemical, and biological behavior of the soil, not much is known about the movement and translocation of the elements in it. One of the fundamental objects of lysimeters has been the study of the outgo of the important plant food elements, the so-called losses which have troubled the soil fertility worker ever since Liebig promulgated his famous mineral theory. And even before Liebig's time the lysimeter was used to evaluate the principle of plant growth by checking and analyzing the leachings.

The comparatively slow progress made in constructing the picture of what is going on in the soil has been due to the inadequate appreciation of what a soil is, its complex constitution and make-up, and its behavior as a natural unit. Lysimeters of the filled-in type have been of limited help in unraveling the secrets of the movement of soil constituents. Their artificial make-up precludes the use of data obtained on leachings from them for the interpretation of phenomena which occur in a natural soil.

With the introduction of the new type of lysimeters at the New Jersey station (3) adopted for the study of soils in their natural habitus a promising approach has been made toward obtaining leachings which represent the natural behavior of the soil.

CONDUCTIVITY AND REACTION OF LEACHINGS[3]

A_1 horizon

Soil reactions hinge on the activity of the inorganic and organic electrolytes formed in the process of decomposition of the mineral and organic constituents of the soil by chemical and biological agencies. The inorganic electrolytes as

[1] ERRATUM: In the author's paper "Lysimeter Studies: I. Moisture Percolation through the Soil Profile, "*Soil Sci.* (1932) 34: 142, reference (9) should read: *Gemmerling, U. V.* 1922 *On the moisture regime of podzol soils on the basis of data from lysimeters (Russian). Trudui Moskor. Sel'sko-Khoz. Oblas. Stan. Bul. 1: 88–95.*

[2] Journal Series paper of the New Jersey Agricultural Experiment Station, department of agronomy.

[3] For a detailed description of the lysimeter set-up, methods of sampling and analyses the reader is referred to the first paper of this series (3).

a rule are highly dissociated in water and exhibit a high conductivity, especially with an increase in concentration, as measured by the Kohlrausch apparatus. Organic acids in the presence of their salts behave in a similar manner. Conductivity measurements may therefore serve as indexes of the concentration of solutes present.

Tables 1 and 2 present in terms of reciprocal ohms the conductivity of the leachings from the various horizons in the soil profile for two consecutive

TABLE 1

Conductivity and reaction of leachings for 1929–1930

NUMBER OF LEACHING	DATE OF COLLECTION	LYSIMETER UNDER HORIZON A_1			LYSIMETER UNDER HORIZON A_2			LYSIMETER UNDER HORIZON B_1		
		Quantity collected	Conductivity*	Reaction	Quantity collected	Conductivity	Reaction	Quantity collected	Conductivity	Reaction
		liters	*mhos*	*pH*	*liters*	*mhos*	*pH*	*liters*	*mhos*	*pH*
	1929									
1	6/22	1.065		4 8	0 165		4 8			
2	6/24	0.510		4.8	0 00					
3	7/30	1.400		4.8	0.00					
4	8/15	0.755		4.8	0.00					
5	9/9	4 740	114×10^{-6}	4.8	2.400	121.8×10^{-6}	4 8	0 050	149×10^{-6}	5.4
6	9/18	0.470		4.8	0.00					
7	10/3	3 440	159×10^{-6}	5.4	0 210	141.1×10^{-6}	5.6			
8	10/23	0 930	257×10^{-6}	6 2	0.00					
9	11/5	0.390	243×10^{-6}	6 4	0 00					
10	11/20	0.135	251×10^{-6}	6.4	0.00					
11	12/20	1.180	155×10^{-6}	4 8	0.090	232×10^{-6}	5.0			
	1930									
12	2/18	3.435	102×10^{-6}	4 6	1.265	112×10^{-6}	4.8			
13	3/11	0.250	119×10^{-6}	4 8						
14	5/17	0.100	121×10^{-6}	4.6						
15	6/11	2.200	78×10^{-6}	4.8	1.395	135×10^{-6}	4 8	0.050	168×10^{-6}	5.4

Total leachings 21.000.

* The conductivity has been determined by the Kohlrausch apparatus at a temperature between 22 and 23°C.

years. Figure 1 gives a graphic illustration of the tendencies in the conductivity of the leachings from A_1 for the two consecutive years.

There is a remarkable similarity between the two curves for 1929–1930 and 1930–1931, which points to a yearly periodicity in the type and content of electrolytes released in the soil. The conductivity takes an upward turn in the fall just about the time when the leaves are heavily shed. It indicates that at that time the soil becomes enriched with highly dissociated inorganic electrolytes. Apparently the soluble inorganic salts present in the freshly fallen leaves are washed out by the fall rains, augmenting the salt content of

TABLE 2

Conductivity and reaction of leachings for 1930–1931

NUMBER OF LEACHING	DATE OF COLLECTION	LYSIMETER UNDER HORIZIN A_1			LYSIMETER UNDER HORIZON A_2			LYSIMETER UNDER HORIZON B_1			LYSIMETER UNDER HORIZON C		
		Quantity collected	Conductivity	Reaction	Quantity collected	Conductivity	Reaction	Quantity collected	Conductivity	Reaction	Quantity collected	Conductivity	Reaction
		liters	*mhos*	*pH*	*liters*	*mhos*	*pH*	*liters*	*mhos*	*pH*	*liters*	*mhos*	*pH*
	1930												
16	7/10	1.300	94×10^{-6}	4 8									
17	7/23	1.300	83×10^{-6}	4 8									
18	8/16	0.080	90×10^{-6}	4.8									
19	8/24	3.350	161×10^{-6}	4 8									
20	9/17	0.350	352×10^{-6}	6 1									
21	10/16	0.325	345×10^{-6}	6 4									
22	10/29	0.608	344×10^{-6}	6 4									
23	11/6	1.455	213×10^{-6}	5 4									
24	11/19	2 420	200×10^{-6}	4 8									
25	12/30	4 090	167×10^{-6}	4 6	1 870	152×10^{-6}	4.6	0 160	135×10^{-6}	6.8	0.355	113×10^{-6}	7.4
	1931												
26	2/19	4.100	151×10^{-6}	4.6	1 200	120×10^{-6}	4 8	0 050	123×10^{-6}	4.9	0.080	103×10^{-6}	4.9
27	3/9	1.770	118×10^{-6}	4.8	0 250	123×10^{-6}	4.8						
28	3/30	3 305	117×10^{-6}	4.8	0 910	203×10^{-6}	4 6	0 025	180×10^{-6}	5.0			
29	4/2	0.665	126×10^{-6}	4 6									
30	4/12	0.505	143×10^{-6}	4 8									
31	5/15	0.700	128×10^{-6}	4 8									
32	5/24	0.475	140×10^{-6}	4.8									
33	6/11	1.950	145×10^{-6}	4 8				0 040	607×10^{-6}				
34	6/18	0.475	155×10^{-6}	4.8	0.045	142×10^{-6}	4.8	0.020	74×10^{-6}				

Total leachings 29.223.

the leachings which is manifested in the conductivity. The high conductivity does not last long. Soon a drop takes place. It is of interest that the rise in conductivity corresponds to low percolation, which means a high concentra-

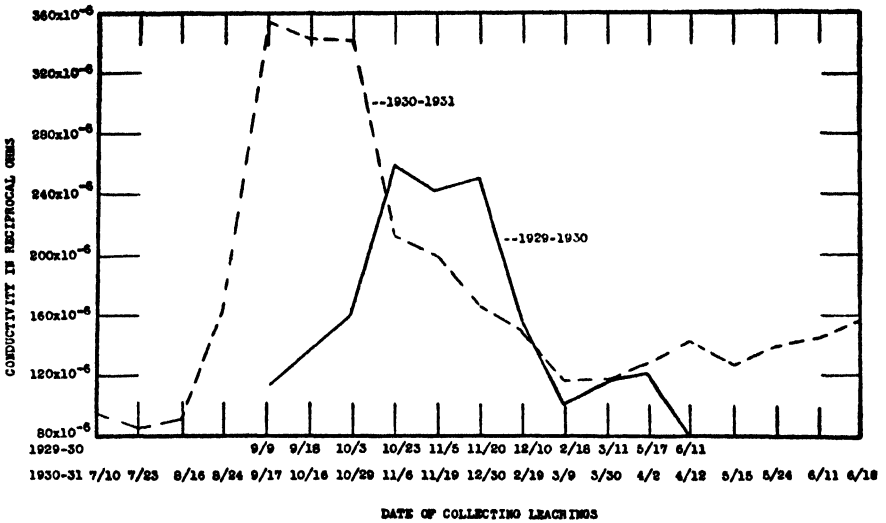

FIG. 1. CONDUCTIVITY CHANGES IN LEACHINGS FOR 1929–1930 AND 1930–1931

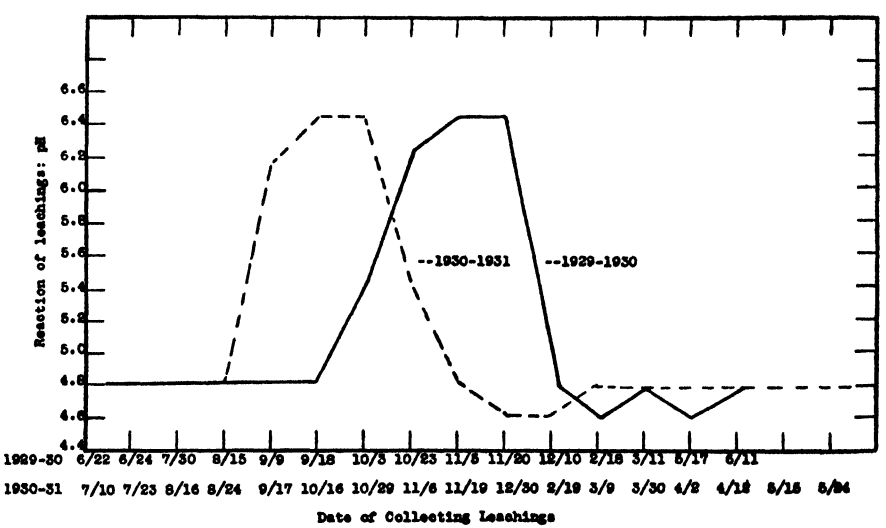

FIG. 2. REACTION CHANGES IN LEACHINGS FOR 1929–1930 AND 1930–1931

tion of solutes, whereas the drop in conductivity corresponds to a high percolation, which means a low concentration of solutes.

Figure 2, illustrating the relation of the H-ion concentration of the leachings in A_1 to the time of collecting them, shows that the rise in pH corresponds to

the rise in conductivity and similarly the drop in pH corresponds to the drop in conductivity.

The rise in pH is due to an increase in OH ions and a suppression of the H ions, which means that the salts released from the freshly fallen leaves consist largely of bases: K, Na, Ca, Mg. Lack of material precluded any determinations for the alkali bases, but the data in tables 3 and 4 clearly show that just during the period of the rise in conductivity the concentration of the alkaline earth bases goes up.

A significant fact about the pH values of the leachings from A_1 is the similarity in periodicity and in the parallelism of the curves (compare figure 2 with figure 1) to those of the conductivity. Of course, the increased base content of the leaching is responsible for the rise in the pH values. A similar observation on the increase of the pH in soils during the fall has been made by Fehér (1) in his study on the microbiological activities in forest soils. There is another still more significant fact about the pH of the leachings: except for the brief period during the fall the pH of the leachings is practically constant—between 4.6 and 4.8. And yet the pH of the soil material in the A_1 horizon ranges between 5.1 and 5.2. No explanation suggests itself at this time to account for this peculiarity.

A_2, B_1, and C horizons

Because of the lack of any appreciable number of leachings from A_2, still fewer from B, and only two from C, the few conductivity measurements do not warrant any definite conclusions. It seems that the tendency is for the conductivity in A_2 to be about the same as in A_1, to increase in B, and to decrease in C. This, however, is not at all true for the leachings of 1930–1931.

From theoretical considerations about the podzol process of soil formation there should be more electrolytes under A_2 than under A_1 thereby tending to increase the conductivity. On the other hand the leachings from B should be lower in electrolytes because of the new formations in this horizon. But the soil under consideration is not a mature podzol and therefore some of the reactions might not be typical of a podzol. With the accumulation of more data on the leachings some of the puzzling points might be cleared up and thereby reveal new facts about the podzolization process.

As to the pH values of the leachings from these horizons, as shown in tables 1 and 2, it is clear that an increase takes place in B, where the bases are retained—permanently or temporarily—because of the characteristic property of this horizon to accumulate basic materials and to be less unsaturated than the horizons above.

MOVEMENT OF SOIL CONSTITUENTS THROUGH THE PROFILE

A_1 horizon

The method of analyses pursued is as follows: 500 to a 1,000 cc. of the leachings are evaporated in platinum dishes, and the residue is dried at 105°C.

TABLE 3

Chemical constituents which percolated through A_1 (18 cm. from the surface) in 1929–1930

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	LEACHINGS	TOTAL SOLIDS AT 105°C. IN		LOSS ON IGNITION			Ca IN		Mg IN		Cl IN		S IN		R_2O_3 IN		SiO_2 IN	
			100 cc.	Total leachings	100 cc.	Total leachings	Per cent	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1929																		
1	6/22	1 065	14 5	154 3	6 8	72 42	46 9	0.696	7.41							0.80	8.520	0.20	2.130
2	6/24	0 510	11 0	56 1	4 6	23 46	41.8	0 835	4 26							0.16	0.820	0.24	1.224
3	7/30	1 400	20 7	289 8	10.16	142 24	49.0	1 153	16 148			0 693	9.696	1.632	22.848	0 48	6.72	0.48	6 720
4	8/15	0.755	15.0	113 25	5 80	43 79	38 6	0 994	7 505			0 396	2.990	0.604	4.560	0 333	25.142		
5	9/9	4 740	12.9	611.46	5 72	271 13	44 3	0 736	34 886	0 205	9.717	0 3564	16.893	1.086	51.476	0 380	18.012	0 24	11.376
6	9/18	0 470	10.1	47 47	3 40	15 98	33 6	1 10	5 17			0 2474	1.163	1.099	5 165	0 956	4.492	0.18	0.846
7	10/3	3 440	14 82	509 80	5.29	181 97	35 6	1.183	40 695			0 6185	21.276	1.588	54.627	0.72	24.768	0 373	12.831
8	10/23	0.930	20 4	189.72	7.20	66 96	35 2	1.530	14.229			0.841	7.821	1.840	17.112			0.466	4.334
9	11/5	0.390	21 78	84 94	8 78	34 24	40 3	1.60	6.240									0.610	2.379
10	11/20	0.135	No determinations made																
11	12/20	1.180	15.00	177 0	5 15	60 77	34 3	0 74	8 732	0 437	5.157					0.23	2.714		
	1930																		
12	2/18	3.435	11 02	378 54	2.285	78 49	20 7	0 23	7.90			0 24	8.244					0.161	5.53
13	3/11	0.250	No determinations made																
14	5/17	0 100	No determinations made																
15	6/11	2 200	13 68	300 96	6 06	133 32	44 2	0 17	3 74	0 205	4 51	0.18	3 960			0.20	4.40	0.30	6 6
1. Total.....		21.000	180.90	2,913 34	71 24	1,124 77		10 967	156 915	0 847	19.384	3 572	72.043	7.849	155.788	4.259	95 588	3.250	53.97
2. Average per 100 cc.*.....			15 08	14.21	5 93	5 49		0.914	0 764	0 282	0 272	0.446	0.38	1.308	1.327	0.473	0.606	0.325	0.290
3. Calculated totals†...			2,984 10		1,152 9			160 44		57.12		79.80		278.67		127.26		60.90	
4. Total per acre in grams‡..			164,125 5		63,409.5			8,824.2		3,141.6		4,389.0		15,326 85		6,999.3		3,349.5	
5. Total per acre in pounds.....			361 5		139 6			19.4		6 91		9.6		33.76		15.41		7.38	

* The average per 100 cc. under the columns "total leaching" was calculated by dividing the respective total constituents by the respective total leachings on which determinations were made.

† To obtain the calculated totals on all the 15 leachings the average per 100 as calculated was multiplied by 21 liters—the total leachings for the year.

‡ The total per acre was calculated by multiplying the figures in 3. by 55,000, the area of the lysimeter being equal to $\frac{1}{55,000}$ acre.

TABLE 4

Chemical constituents which percolated through A_1 (18 cm. from the surface) in 1930–1931

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	LEACHINGS	TOTAL SOLIDS AT 105°C. IN		LOSS ON IGNITION			Ca in		Mg IN		Cl IN		S IN		R_2O_3 IN		SiO_2 IN	
			100 cc.	Total leachings	100 cc.	Total leachings	Per cent	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1930																		
16	7/10	1.300	18 90	245 70	12 42	161.42	65 6	0 52	6.76	0 177	2.301					0 48	6.24	0.42	5.46
17	7/23	1.300	12 20	158 60	7 06	91 78	57 8	0 66	8 58	0 161	2.093					0.50	6.50	0.46	5.98
18	8/16	0.080	No determinations made																
19	8/24	3.350	22.08	739 68	12 08	404 68	54 7	0 46	15.41	0 275	9 213	0.12	4 020	1 35	39 225	0.56	18.76	0.26	8.71
20	9/17	0.350										0.06	0 210	3.20	11.200				
21	10/16	0 325																	
22	10/29	0.608	46 20	280 90	23 66	143 85	51 2	1 40	8.51	0 728	4 426	0 16	0.973	2 65	16.112	0.53	3.24	0.13	0.81
23	11/6	1.455	33 04	480.73	15 00	218 25	45 3	0.88	12 80	0.534	7.769	0 145	2 110	2 70	39 285	0.50	7 27	0.12	1.75
24	11/19	2.420	23 60	571 12	7 88	190 69	33 3	0 82	19 84	0 423	10 237	0.100	2 420	1 80	43.560	0 70	16 94	0 28	6.78
25	12/30	4 090	12.30	503 07	5 00	204 45	40.6	0 74	30 27	0.334	13.661	0 600	24 540	0 96	39 264	0.73	29.86	0.16	6.54
	1931																		
26	2/19	4.100	10 60	434 60	4 14	169 74	39.0	0 73	29.93	0 292	11.97	0 540	22 14	1 23	50.43	0 66	27.06	0.20	8.20
27	3/9	1.770	10 18	180 19	3 65	64.61	35 8	0 75	13.28	0 301	5 328	0 580	10 27	1.15	20 355	0.706	12 50	0.106	1.88
28	3/30	3 305	9 42	311 33	3.50	115 68	37 1	0 60	19.83			0.580	19 169	0 88	29 084	0.610	20.16	0.070	2.31
29	4/2	0.665	9 47	62 98	2 52	16 76	26 6	0 63	4.19	0.308	2 048			1 40	9.31				
30	4/12	0 505	10 22	51 61	2 62	13 23	25 6	0 67	3.38	0 350	1.767								
31	5/15	0 700	10 02	70 14	3.72	26 04	37.1	0 31	2.17	0 131	0.917							0.240	1.68
32	5/24	0 475	No determinations made																
33	6/11	1.950	15 80	308 10	9 60	187 20	60 7	0 56	10.92	0 245	4.777							0.213	4.15
34	6/18	0 475	No determinations made																
1. Total.		29 223	244 03	4,398 75	112 85	2,008 38		9 73	185 87	4 259	76 507	2.885	85 852	17 32	297.825	5.976	148 53	2.659	54.25
2. Average per 100 cc.*.			17 4	15 9	8 06	7 3		0 695	0 675	0 328	0 316	0 3616	0 400	1 732	1.346	0 598	0 627	0.222	0.206
3. Calculated totals†...				4,646 46		2,133 3			197 255		92 345		116 892		393 341		183.228		60.199
4. Total per acre in grams‡....				255,555 3		117,331 5			10,849 03		5,078.98		6,429 06		21,633.75		10,077.54		3,310.95
5. Total per acre in pounds.....				562 8		258.4			23 8		11.18		14 16		47.6		22.19		7.29

*, †, ‡ For explanatory legends see table 3.

and then heated to cherry red for loss on ignition. The ash is taken up with HCl, the SiO_2 is stabilized, and the rest of the constituents are determined in the regular way. The data in tables 3 and 4 give a detailed account of the constituents released from the A_1 horizon. An analysis of the data offers many clues as to what is going on in the A_1 horizon, that part of the soil body which in a large measure determines the behavior of the other parts. For this horizon is the potential source of the various constituents which enter into the "blood circulation" (soil solution) of the soil body.

A discussion of each one of the constituents will help to clarify the intricate reactions in their relation to the leachings, seasonal variations, and each one constituent.

Total solids and loss on ignition. The quantity of total solids—in pounds per acre—which percolated through A_1 in 1930–1931 is close to 56 per cent higher than in 1929–1930. This might not be surprising in view of the larger total leachings in 1930–1931—29.223 liters against 21.0 liters in 1929–1930. On the other hand one might have expected that an increase in leachings would dilute the solids, but this was not the case. The average concentration of solids per 100 cc. in the leachings of 1930–1931 increased by 11.8 per cent—15.9 against 14.21 mgm. per 100 cc. of leachings. The cause of such a behavior is, of course, problematic. It will be recalled (3) that the rainfall in 1929–1930 was higher than in 1930–1931—37.26 and 34.14 inches respectively. But the effective precipitation—the amount of rainfall after which leachings percolated through the horizon—was higher in 1930–1931 than in 1929–1930—24.81 and 22.47 inches respectively—, which accounts, as pointed out (3), for the higher total leachings. Evidence was presented to prove that the state of the colloids is probably responsible for the difference in percolation. Whether this has anything to do with the higher concentration of total solids in the leachings of 1930–1931 is not at all clear.

A qualitative analysis of the total solids offers a clue to the probable cause of the apparently anomalous quantitative results. An examination of the percentage of loss on ignition on the total solids reveals the fact that it is higher in 1930–1931 than in 1929–1930: 45.9 and 38.6 per cent respectively. There can be no other reason for such higher loss on ignition except that there was more organic matter in the total solids of the second year's leachings. This means that more organic matter went into circulation in 1930–1931 carrying, of course, also large amounts of inorganic constituents, for humifying and mineralizing reactions go hand in hand. Apparently the environmental conditions for microbiological activities in the soil were more favorable in 1930–1931 than in 1929–1930. There is another way of looking at this qualitative difference of the total solids. As pointed out, they are 56 per cent higher in 1930–1931 than in 1929–1930, but if we calculate the total ash (by subtracting the loss on ignition from the total solids) we find it is only 37 per cent higher, thus 19 per cent increase of the total solids is to be ascribed to the increase in organic matter.

With the increase in quantity of total solids came also the increase in concentration of solutes per unit of solution, as pointed out. And a glance at the columns on the total solids per 100 cc. of leachings shows that this increase is more apparent during the months of October and November. This will help to explain the higher peak in the conductivity curve (fig. 1) of the leachings of 1930–1931 even though the type of curve has remained the same as for the leachings of 1929–1930. Such behavior is in harmony with the conductivity laws established by Kohlrausch.

Calcium and magnesium. The release of bases from the A_1 horizon is true for every type of soil formation. It is the movement and translocation of these bases in the soil profile that differentiate the types of soil formation in the temperate arid and semi-arid regions from the temperate humid region. In the podzol process of soil formation the movement and translocation of these bases are more intense and result in actual losses from the soil body.

A comparison of the losses of bases shows that during the second year the amount of Ca in the leachings was 22.6 per cent higher than during the first year (the difference between 23.8 and 19.4 pounds for the respective years) and the amount of Mg lost was 61.7 per cent higher (the difference between 11.18 and 6.91 pounds). The reason for the relatively higher loss[4] of Mg, as compared with Ca, is not clear.

Both Ca and Mg show the same tendency to increase in concentration during the summer and fall, attaining a maximum concentration in October and November and dropping off during the winter and spring. It may not be out of place to recall again that the highest concentration of the bases corresponds to the highest conductivity and pH. There seems to be some correlation between the quantity of leachings and the concentration of Ca in the leachings. This suggests that we are dealing with a salt of a low solubility product and it might be $CaSO_4 \cdot 2H_2O$.

An interesting point to be noted is that the average concentration of Ca per 100 cc. is higher in 1929–1930 than in 1930–1931. The reverse is true for the Mg.

Chlorine and sulfur. There seems to be no consistent regularity in the chlorine concentration of the leachings at the various seasons of the years. Apparently the chlorine content depends on the prevailing atmospheric condition over the Atlantic seaboard. Some rains being more chlorine, some less.

The high sulfur content in the leachings in the form of sulfates is indicative of the rapid conversion of sulfur compounds into sulfates. Unquestionably it is the oxidation of the sulfur in the organic matter that augments the leachings with sulfates. The higher organic matter content in the leachings of the second year is probably responsible for the higher sulfur outgo. There is a higher concentration of sulfates in the leachings during the fall than at any other season of the year. This is clearly brought out in the sulfur data on the leachings of the second year.

[4] Actually these losses can not be considered as such, as will be shown presently. In reality these are the quantities which percolated through the A_1 horizon.

Sesquioxides and silica. There is no other set of compounds released in the podzol process of soil formation which characterizes the process as the sesquioxides do. They move through the profile in the colloidal state protected by the negatively charged humus compounds, as pointed out elsewhere (2). The leachings offer an opportunity to follow up the movement and translocation of the sesquioxides.

In presenting the data on the sesquioxides it is well to point out that no provisions have been made for the evaluation of the PO_4 and Ti ions which come down with them.

Considering the sesquioxides as they are, we note that their release fluctuates from year to year. With the increase in total solids during the second year there was a corresponding increase of the R_2O_3 constituents. They also show a tendency to increase in concentration in the leachings during the fall, but not in such striking manner as the other constituents do. There are other factors inherent in the nature of colloids which might control the movement of the sesquioxides. One of them is the state of aggregation of the colloids. It is known that after heavy rains the soil colloids become more highly dispersed and therefore more mobile. The mechanism of this reaction is simple: the electrolytes are removed by the rain water from the surface horizon and moved downward. The absence of the electrolytes tends to peptize the colloids, and naturally the degree of dispersion increases, as a result of which the diffusion coefficient increases. An examination of the data in tables 3 and 4 on the concentration of R_2O_3 in the leachings shows that after heavy percolation, which follow heavy rainfall, it increases. This, however, is to be noted: the data also show that usually the periods of high R_2O_3 concentration are also periods of high concentration of other constituents. Theoretically there is the possibility of retardation of the R_2O_3. A preliminary check-up has shown that the electrolytes seem to appear with the first few hundred cubic centimeters of leachings, whereas the R_2O_3 concentration begins to increase after that. More data are to be accumulated before anything more definite might be said about this phase.

There is no positive correlation between the loss on ignition (organic matter) and the sesquioxides. If anything, the data show that a larger loss on ignition corresponds to a lower sesquioxide content. This might appear as a contradiction to the prevailing idea that the sesquioxides move with the humus complexes. As a matter of fact it is not known whether an excess of humus is conducive to a higher mobility of the R_2O_3 and, perhaps, the smaller amount present was sufficient for the protective action exerted on the R_2O_3.

It is well to associate the movement and translocation of the R_2O_3 with the fundamental truth that in the circulation of the elements through the soil profile the sesquioxides do not reappear in the accumulative A_0 layer as the case is with other constituents. Plants take up little Fe and still less, if any, Al. It is the absolute loss of the eluviated R_2O_3 that is so significant in the process of podzolization. This influences the arrangement and distribution of the other soil constituents.

Closely related to the behavior of the sesquioxides is the silica. On the face of it, both exist in the leachings in colloidal state as sols and, notwithstanding their being oppositely charged electrically, they do not coagulate. From the work of Simakov (7) it would seem that since 1 mole of SiO_2 coagulates from 0.577 to 1.302 moles of Al_2O_3 or 0.290 to 0.363 moles of Fe_2O_3, the combination of the two should coagulate when the $\frac{SiO_2}{R_2O_3}$ ratio is from 1.2 to 2.3. In table 5 a few determinations on the $\frac{SiO_2}{R_2O_3}$ are reported. It shows that the ratio varies from 1.04 to 1.92, which is within the coagulation power of the SiO_2, and yet the colloids exist in the sol state. Apparently other factors such as the pH and presence of electrolytes hinder the coagulation.

TABLE 5

Silica and sesquioxides in leachings from A_1

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	LEACHING	SiO_2 IN 100 CC.	Fe_2O_3 IN 100 CC.	Al_2O_3 IN 100 CC.	Al_2O_3 AND Fe_2O_3 IN 100 CC	$\frac{SiO_2}{R_2O_3}$	REACTION
		liters	*moles*	*moles*	*moles*	*moles*	*molar ratio*	*pH*
	1929–30							
3	7/30	1.400	$7\ 96\times10^{-6}$	0.99×10^{-6}	3.14×10^{-6}	$4\ 13\times10^{-6}$	1.92	4 8
5	9/9	4 740	3.98×10^{-6}	$0\ 496\times10^{-6}$	$2\ 945\times10^{-6}$	$3\ 441\times10^{-6}$	1.15	4 8
7	10/3	3.440	$6\ 18\times10^{-6}$	1.99×10^{-6}	$3\ 93\times10^{-6}$	$5\ 92\times10^{-6}$	1.04	5 4
	1930–31							
17	7/23	1.300	7.62×10^{-6}	0.98×10^{-6}	$3\ 34\times10^{-6}$	4.32×10^{-6}	1.76	4.8

A significant point to be noted in table 5 is the low $\frac{SiO_2}{R_2O_3}$ ratio in leaching 7 where an increase in pH occurred. This is in line with the findings of Mattson (6), who shows how the $\frac{SiO_2}{R_2O_3}$ ratio decreases as the pH increases and vice versa. More determinations on this important relationship between the sesquioxides and the silica must be made before the entire significance of it is clearly brought out.

A_2 horizon

Tables 6 and 7 present the data on the constituents that passed through a depth of 42 cm. A comparison of the quantities of constituents which leached through in the 2 years under consideration shows that the first year gave more than the second in spite of the fact that the amounts which leached through A_1 were just the reverse: during the second year more solids leached through, as shown in tables 3 and 4. This means that during the first year less of the substances which reached the A_2 horizon were retained by it, whereas more of the constituents from the second year's leachings were retained by the A_2

TABLE 6

Chemical constituents which percolated through A_2 (42 cm. from the surface) in 1929–1930

LABORATORY NUMBER OF LEACHING	DATE	LEACHINGS	TOTAL SOLIDS AT 105°C. IN		LOSS ON IGNITION			Ca IN		Mg IN		Cl IN		S IN		R_2O_3 IN		SiO_2 IN	
			100 cc.	Total leachings	100 cc.	Total leachings	Per cent	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1929																		
1	6/22	0.165																	
5	9/9	2.400	10.10	242 40	2 90	69 60	28 7	0 855	20 52	0.188	4.512	0.495	11 880	1 016	24 384	0 30	7.200	0.28	6.72
7	10/3	0.210	No determinations made																
11	12/20	0.090	No determinations made																
	1930																		
12	2/18	1.265										0 620	7.119	1.000	12 650				
15	6/11	1 395	8.12	113 27	3 92	54 68	48 2	0.60	8 37	0 262	3.655	0.570	7.952	1.100	15 345	0 30	4.185	0.34	4.743
Total*.....		5.525	18 22	355 67	6 82	124 28		1.455	28 89	0 450	8 167	1 685	26.951	3 116	52.379	0.60	11.385	0.62	11.463
Average per 100 cc.*....			9.11	9 37	3 41	3 27		0 7275	0 761	0 225	0 215	0.562	0.532	1 038	1.035	0.30	0 30	0.31	0.302
Calculated totals*.....			517.693		180.668			42 045		11 879		29 393		57.184		16.575		16.69	
Total per acre in grams.....			28,473.1		9,936 7			2,312 5		653 34		616 6		3,145.1		911.63		917.95	
Total per acre in pounds.....			62.71		21.88			5.1		1 43		3 56		6 9		2.01		2.02	

* For methods of calculating averages and totals, see table 3 on A_1 leachings.

TABLE 7

Chemical constituents which percolated through A_2 (42 cm. from the surface) in 1930–1931

LABORATORY NUMBER OF LEACHING	DATE	LEACHINGS	TOTAL SOLIDS AT 105°C. IN		LOSS ON IGNITION			Ca IN		Mg IN		Cl IN		S IN		R_2O_3 IN		SiO_2 IN	
			100 cc	Total leachings	100 cc	Total leachings	Per cent	100 cc	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings	100 cc	Total leachings	100 cc.	Total leachings	100 cc.	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm*		*mgm.*	*mgm*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1930																		
25	12/30	1.870	10 92	204 20	3 80	71 06	34 3	1 06	19 82	0 393	7 35	1.13	21.13	1 00	18.7	0.72	13.46	0 28	5.24
	1931																		
26	2/19	1.200	7.48	89.76	2.08	24 96	27 8	0 74	8 88	0 262	3 144	0 44	5 28	1 05	12 6	0 58	6 96	0.24	2.88
27	3/9	0 250										0 60	1.50	1 10	2.75				
28	3/30	0.910	7 20	65 52	2 01	18 29	27 9	0 82	7 46	0 302	2 75	0 80	7.28	2 00	18 20			0.28	2.55
34	6/18	0 045	No determinations made																
Total*.....		4.275	25 60	359 48	7.89	114 31		2 62	36.16	0 957	13 244	2.97	35.19	5 15	52.25	1.30	20.42	0 80	10.67
Average per 100 cc.*....			8 53	9 03	2 63	2 87		0 873	0 908	0.319	0 332	0 742	0 83	1.29	1 24	0 65	0.665	0 266	0.268
Calculated totals*..			386 03		122 69			38 82		14.19		35 48		53 01		28 43		11.46	
Total per acre in grams*....			21,231 7		6,747 9			2,135 1		780 5		1,951 4		2,915 6		1,563 7		630 3	
Total per acre in pounds*..			46 7		14 8			4 7		1.7		4 3		6 4		3 4		1.3	

* For methods of calculating averages and totals, see table 3 on A_1 leachings.

horizon. The significance of the rôle of the A_2 horizon in the movement and translocation of soil constituents will be more clearly brought out in the discussion following.

Total solids and loss on ignition. The increased quantity of total solids in 1929–1930 as compared with 1930–1931, corresponds to the increase in total leachings as shown in tables 6 and 7. Why A_2 percolated more in 1929–1930 than in 1930–1931, notwithstanding that less leachings from A_1 reached it, and why the reverse was true for 1930–1931 has been discussed in a previous publication. In short, it has been pointed out that the state of the colloids control the percolation rather than the total volume of rainfall or leachings.

Just as in the case of the leachings from A_1, the increased percolation also increased the concentration of the total solids—3.27 against 2.87 mgm. per 100 cc.—instead of decreasing it as one might have expected. There is, however, a distinct difference in the loss on ignition—34.8 and 31.7 per cent in A_2 and 38.6 and 45.9 per cent in A_1. This, of course, is due to the retention of organic matter in A_2. Of the total solids which reached the A_2 from A_1 only 17 per cent percolated through A_2 in 1929–1930 and 8.3 per cent in 1930–1931.

Calcium and magnesium. Of the total 19.4 pounds of Ca which reached the A_2 horizon from A_1 during the first year, only 5.1 pounds percolated, i.e. only 26.2 per cent was lost, if we consider the substances that pass a depth of 42 cm. (about 16 inches) as such. During the second year of a total of 23.8 pounds 4.7 pounds, or 20 per cent, percolated. It is of interest to note that whereas the total solids were 34.7 per cent higher in the leachings of the first year when of the second there was only 8.5 per cent more Ca. The Mg content was even lower, and the same was true for the Cl and R_2O_3. This again points to the fact that the increase in total solids was due primarily to the organic matter.

A comparison of the concentration of Ca per 100 cc. of the leachings in A_1 and A_2 for the corresponding leaching numbers shows that in 1929–1930 (see leachings 5 and 15) it increased in A_2. A similar tendency is to be noted in 1930–1931 (see leachings 25 and 28). Unfortunately there are not on hand many more determinations which might throw more light on the movement of this all-important cation. But as it stands, we might conclude that there is a tendency for the increased quantities of Ca in A_2 to leach away. One must not forget that it is this horizon which serves as the feeding grounds for the forest vegetation, and how much of the mineral substances are taken out in this manner is not definitely known.

Chlorine and sulfur. The inconsistency noted in the Cl content of the A_1 leachings is also true for the A_2 leachings. In 1929–1930 more than 37 per cent and in 1930–1931 more than 30 per cent leached away. From these data it seems that a considerable amount of Cl remains behind in A_2. How much of it is used up by the forest vegetation to go again into circulation is not known.

Large quantities of sulfur remain behind in A_2, but a good portion of it is used up by the forest vegetation to be brought again into circulation.

Sesquioxides and silica. In mature podzols the A_2 is characterized by its low R_2O_3 content. Thus in following up the process of podzolization—as this could be done in the lysimeter studies reported—one should expect a high outgo of R_2O_3 from A_2. From the meager data on hand for the 2 years this is not substantiated to any appreciable extent. There is an indication that the concentration of R_2O_3 does increase in the leachings of the A_2 horizon. As the data stand, it would seem that the soil under consideration is only slowly podzolized. Morphological and chemical evidences to this effect are on hand and will be published in some other connection.

Nothing definite may be said about the SiO_2. The difficulty in measuring such small quantities of SiO_2 makes small numbers of determination rather doubtful, and the few leachings obtained from A_2 did not permit any larger number of determinations to be made.

GENERAL DISCUSSION

In the foregoing pages an attempt has been made to account for the constituents which move through the soil profile from the A_1 horizon to A_2 and from there to the B horizon. Nothing has been said about the upward movement of the various constituents by the forces of capillarity. It is possible that some of the obscure results might find an explanation just in this upward movement, which takes place especially through A_2 toward the bottom part of A_1. This point needs to be investigated.

An important point to be kept in mind in connection with the analyses presented is that the quantitative aspect of the data hinges on the assumption that the direction of the leachings through the horizon of eluviation is vertical. In the first paper of this series (3) on the percolation of moisture through the soil profile evidence has been presented to justify this postulate. It has been pointed out that in the open, practically structureless A horizon there are no obstructions for the water to move in a vertical direction. Only when the moisture reaches the compacted B horizon it begins to diverge from its vertical course and begins to move also in a horizontal direction following hollow root paths, cracks, and burrows of animals and insects.

The 2 years for which data are available were rather abnormal with respect to rainfall. It was below normal: 10 inches in 1929–1930 and 13.12 inches in 1930–1931. Perhaps this rainfall shortage is responsible for the shallow percolation. Under normal rainfall conditions we may get a deeper penetration. This remains to be seen. The fact is that the leachings from B and C are erratic and scant and because of that, very few determinations are available. As a matter of fact the data on hand seem to indicate that most of the constituents which passed the A_2 horizon were retained by the B horizon. Only 1 per cent of the leachings passed through the B horizon and about 2 per cent through the C. Thus a very small proportion of the constituents from the soil under consideration went to the ground waters during the 2 years.

The foregoing discussion invites a comparison of the removal of constituents

as shown by the new type of lysimeters and that of the old one. The Cornell lysimeter data (4, 5) offer detailed figures, and in table 8 a summary has been made. Since no means are available at present to trace the exact movement of the constituents after they pass the A_2 horizon the figures on this horizon are given in table 8 beside the Cornell data.

The remarkably wide differences in the removal of constituents from the soil as shown by the two methods—old type and new type of lysimeters—make one wonder whether the results are reliable. A closer analysis of the data reveals some interesting facts of importance in soil fertility studies and soil in general, to the end of which lysimeter equipments must serve.

According to the figures of Lyon and Bizzell (4) the weight of dry soil—which makes up the first foot in the lysimeters—per acre foot is 3,645,000

TABLE 8

Average annual removal of various constituents from the soil, as indicated by the Cornell and New Jersey lysimeters

(In pounds per acre)

CONSTITUENTS	FROM CROPPED TANKS* BY:			FROM UNCROPPED TANK	FROM THE NEW JERSEY LYSIMETERS
	Drainage	Crop	Total	Drainage	Drainage†
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
Total solids	955 0	?	955.0	2,223.9	54.7
Ca	177.1	13.2	190.3	364 0	4.9
Mg	33.8	7 4	41 2	60.2	1 6
S	35 5	10 4	45 9	44 0	6 7
SiO_2	32.4	?	32 4	48 6	1 66

* The data presented are for the first 5 years (the same is true for the 15-year period) on the cropped tanks 3, 5, and 6 and on the uncropped tank 4. All tanks received two manure applications at the rate of 10 tons per acre during the period. Compiled from N. Y. (Cornell) Agr. Exp. Sta. Mem. 12 (4).

† Based on the leachings from the A_2 horizon; average of 2 years.

pounds with a 0.34 per cent CaO content, which gives a total of CaO equal to 12,393 pounds. An acre foot of dry soil which makes up the second foot in the lysimeters weighs 3,827,500 pounds with a 0.28 per cent of CaO, which gives a total of 10,717 pounds of CaO. An acre foot of dry soil which makes up the third foot in the lysimeters weighs 3,999,500 pounds with a 0.49 per cent CaO content, which gives a total of 19,597.5 pounds of CaO. An acre foot of dry soil which makes up the fourth foot of the lysimeters weighs 3,710,000 pounds with a 1.53 per cent CaO content, which gives a total of 56,763 pounds of CaO. Thus 4 acre feet of soil contain a total of 99,470.5 pounds of CaO or 71,050 pounds of Ca. Besides that, 80 pounds of Ca is added once in 4 years in the form of manure, or 20 pounds per year. On the debit side, we have 190.3 pounds of Ca removed yearly. If we subtract from it the yearly addition of

20 pounds we have a net loss of about 170 pounds of Ca per year. Now, if we divide the soil's Ca assets—71,050 by 170 we get the figure 418, which means that in 418 years not a trace of Ca will remain in a column of soil 4 feet deep. The figure given is for the cropped lysimeter tank. If we take the losses from the uncropped tank and calculate as above it would show that in 206 years no lime would remain.

Let us make another calculation. Of the 2,223.9 pounds of total solids lost from the uncropped tank about one-fourth is probably organic matter, which means that 1,717 pounds constitute the mineral portion. Now the weight of the 4 acre feet column of soil is 15,175,000 pounds. Disregarding the organic matter in it and dividing 15,175,000 by 1,717 we obtain the impressive figure of 8,830 years. In other words after this period of time there would be nothing left in the lysimeter tank, or the surrounding landscape would sink 4 feet. Of course, the author is mindful of the absurdity of such a possibility. This could only happen if the proportion of the constituents in the total solids were equal to that in the soil, which is not the case. It is given simply as a curiosity which perhaps should not find a place in a scientific paper.

But let us return to the Ca removal, the calculations of which will stand the mathematical test, except that actually the Ca outgo will undoubtedly slow down because of the inherent property of soil material to form a soil body, which means a differentiation into horizons with the consequent formation of a B horizon which will prevent the washing out of soil constituents and hence prevent the destruction of the soil.

Without further analysis of the impossibility of the losses as reported by the Cornell lysimeter studies the author is inclined to believe that the removal of the constituents from the soil body as indicated by the new type of lysimeters is closer to actual natural conditions. The calculations perhaps lack precision, but they are based on reasonable grounds and are probably not far wrong.

There is this to be remembered: the data presented are based on leachings from forest soils. How much of the inferences will hold true for cultivated soils is, of course, problematic. Another set of this type of lysimeters in cultivated soils would clinch the problem of the movement of soil constituents and its relation to soil fertility—the ultimate aim of our studies.

In our soil fertility practices we strive to maintain the favorable balance of soil constituents—a state naturally maintained in the forests. A knowledge of the conditions in the virgin soils is the fundamental preamble for the methods which shall be profitably applied when the human being enters as a possible factor in the processes of soil formation, i.e., when cultivation begins. Thus far we have attempted to gain knowledge from empirical experimentation without consulting the actual conditions in the natural soil. After all, we cultivate only 6 inches deep and although this has undoubtedly a marked influence on the remaining larger portion of the soil body the tendencies noted in an undisturbed soil will probably hold true, and especially for the rest of the soil body below the plowed layer. There might be some difference in the degree

of these tendencies but generally they will probably remain true. The data presented are therefore of significance and with the evaluation of the variable conditions existing between cultivated and virgin soils they can be used for comparison with data obtained empirically to show the loopholes existing in our old methods.

SUMMARY

Data on the conductivity and reaction of leachings from the new type of lysimeters at the New Jersey station for a period of 2 years show that during the fall there is an increase in concentration of basic salts in the A_1 horizon. This influences the conductivity—a rise during October and November. A similar rise is noted in the pH. There is a definite parallelism between the rise in conductivity and the increase in pH.

There is an increase in the total quantity of total solids in the leaching of the second year in A_1. This is accompanied with a rise in concentration of the solids. The increase in total solid is primarily of an organic nature.

The alkaline earth bases—Ca and Mg in A_1—show a tendency to increase in concentration toward the end of the summer and fall, attaining a maximum in October and November.

The chlorine content of the leachings shows no consistent behavior. Apparently the direction of the winds and the Cl content of the rains govern the quantity of this ion. The S content is fairly high and it is suggested that the rapid oxidation of the organic sulfur compounds is responsible for augmentation of sulfates in the leachings.

The R_2O_3 also increased in concentration during the second year, but not so much as the other mineral constituents. A discussion is presented on the relation of the removal of R_2O_3 to organic matter and mineral substances. The SiO_2 movement follows the R_2O_3 and the $\frac{SiO_2}{R_2O_3}$ ratio varies from 1.04 to 1.92. The low ratio corresponds to an increase in pH which is in line with the findings of Mattson (6).

Of the total solids which reached A_2 from A_1 only 17 per cent percolated through A_2 during the first year and 8.3 per cent during the second year. There is lower loss on ignition in the total solids from A_2 than from A_1 indicating a retention of organic matter in A_2.

The concentration of the mineral constituents—Ca, Mg, Cl, S, and R_2O_3—increased as a rule in the leachings of A_2 as compared with A_1.

A comparison of the constituents removed in the leachings through A_2 with those removed from the Cornell lysimeters shows that from 20 to 40 times as much, depending on the constituent, was removed from the Cornell lysimeters. Calculations made on the assets and outgo of Ca from the soil of the Cornell lysimeters show the improbability of such losses under natural conditions. The abnormal condition of the soil material in the lysimeter tanks is responsible

for such striking losses. It is inferred that the old type of lysimeters is not suitable for full orientation as to what is actually going on in the soil.

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THE ASPERGILLUS NIGER METHOD OF MEASURING AVAILABLE POTASSIUM IN SOIL[1]

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This investigation deals with the *Aspergillus niger* method of measuring the available potassium of soils. Recently this method has received considerable attention, and hence only a brief review of the literature relating to the method for measuring potassium and phosphorus will be given.

As early as 1909 Butkewitsch (2) suggested the use of *A. niger* as a biological indicator of the amount of available potassium and phosphorus in soils. A method in accordance with this was developed in 1909 by Koszeleskii (7) for available phosphorus, and the results obtained were found to agree closely with those obtained by extracting a podzol soil with citric acid and a chernozem soil with oxalic acid. The publications of Koszeleskii are in Russian, and hence are not easily accessible to investigators of other countries.

Apparently unaware of Koszeleskii's work, Pantanelli (18) reported in 1924 on the use of molds for testing the phosphorus needs of soils. He cultured *A. niger*, *A. oryzae*, and *A. flavus* on a sterilized mixture of soil and a nutrient solution containing 10 per cent sucrose and 2 per cent ammonium sulfate. The phosphorus which accumulated in the fungus mycelium was taken as a measure of the available soil phosphorus. He found that *A. niger* and *A. oryzae* gave the best results.

Benecke and Söding (1), in 1928, following the suggestion of Butkewitsch, used *A. niger*, and also other microörganisms, e.g., *Cladosporium herbarum* and *Stichococcus variabilis*. According to their method 50-cc. portions of the culture medium are placed in Erlenmeyer flasks and sterlized, and then to one series KCl is added in concentrations of 0.00025 to 0.002 per cent and in another series, soil sterilized at 150°C. for 15 minutes, is added in amounts from 0.12 to 5 gm. Both series are inoculated with a suspension of *A. niger* spores, and incubated at 35°C. After 6 days, the mycelium of the soil series is compared with that of the KCl series, and in this way conclusions are drawn regarding the available potassium content of the soil. The procedure for phosphorus is similar to that for potassium, except that 0.1 gm. KCl, and phosphorus in 0.002 to 0.0006 per cent concentrations are added to the medium. The authors considered their work to be of a preliminary nature and made no claim to quantitative results. Later, Simakova and Bovschik in 1932 (21) modified the method so as to make possible the obtaining of quantitative results for phosphorus. They avoided the occlusion of soil in the mycelium by bringing the medium to a boil, causing the soil to settle.

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Lohmann (8) in 1931 reported on a method for determining the available potassium and phosphorus in soils which was essentially a modification of the Benecke and Söding method. He placed soil and nutrient solution in flasks, inoculated them with *A. niger*, and after 6 days of incubation placed the pad on a filter. The soluble salts were removed by washing, and the dried pad of soil plus mycelium was weighed. The weight of the soil was subtracted and from the weight of the mycelium the approximate amount of potassium absorbed from the soil was estimated.

Seidel (20) in 1931 recommended a new microbiological method in which yeast is employed as a biological agent to test for available phosphorus, and *Rhizopus arrhiza* to test for available phosphorus, potassium, and calcium. He claims to have obtained good results with *Rh. arrhiza* and urged its use in the determination of soil fertility.

Niklas, Poschenrieder, and Trischler (12, 13, 14, 15) used *A. niger* to determine the available potassium and phosphorus, and Niklas, Poschenrieder, and Frey (11) used the same organism to test for available magnesium. They and others at the Weihenstephan Station carried out experiments on various nutritive and other factors influencing the weight of mycelium of *A. niger* (24, 17, 4, 27, 26). As a result of extensive studies Niklas and Poschenrieder (10) recommended the following nutrient solution and procedure for carrying out the test:

Cane sugar	100 gm.
Citric acid	10 gm.
Ammonium sulfate	6 gm.
Peptone	1 gm.
P_2O_5(1.2152 gm. of $NH_4H_2PO_4$)	0.75 gm.
$MgSO_4$(0 6143 gm. of $MgSO_4 \cdot 7H_2O$)	0.30 gm.
Cu(0 0059 gm. of $CuSO_4 \cdot 5H_2O$)	0 0015 gm.
Zn(0.0044 gm. of $ZnSO_4 \cdot 7H_2O$)	0.0010 gm.
Fe(0.00498 gm. of $FeSO_4 \cdot 7H_2O$)	0 0010 gm.
Water, distilled	1,000 cc.

A 2.5-gm. portion of soil and 30 cc. of the nutrient solution are placed in a 75-cc. Erlenmeyer flask, inoculated with a suspension of spores of *A. niger* and incubated at 35°C. for 4 days. For more exact tests the mycelium is harvested after 6 days' growth instead of 4. The pads are washed and dried, first at 50° to 60°C. for 12 hours, then at 80° to 90°C. for 1 to 2 hours, and finally at 105°C. for 2 to 3 hours. If the weights of the dry pads fall below certain minimums, the need of potash fertilization is indicated. For calcareous soils, certain corrections are made. Their results with *A. niger* compared very favorably with those obtained with the Neubauer seedling method (16, 3).

Kiessling (5) also obtained good results with *A. niger* in determining the available potassium of soils. He found that the growth of mycelium is markedly stimulated by the presence of humic acid (6).

Recently Smith, Brown, and Schlots (22) reported the results of a comparative study of the Niklas *A. niger* and Truog chemical methods for available phosphorus. They found the two methods to check very well. They also concluded that the citric acid present in the medium is responsible for the solution of the soil phosphorus in the *A. niger* method, and the same results could probably be obtained with a simple citric acid extraction of the soil.

EXPERIMENTAL

Preliminary tests with the *Aspergillus niger* method of measuring the potassium deficiencies of soil, carried out according to the procedure of Niklas, excepting that 125-cc. flasks were used and drying of the pads was accomplished at 70° to 90°C. for 12 to 14 hours and then at 105°C. for 2 hours, gave results which in many cases failed to agree with those of a chemical method for re-

placeable potassium recently developed by Volk and Truog (28). Because of this lack of agreement, a number of experiments were made to determine, if possible, the disturbing factors which influence the weight of mycelium, and hence the accuracy of the method.

Comparison of different strains of Aspergilli

Since the weight of the pad is supposed to bear a direct relation to the available potassium of the soil, tests were made with a soil low in replaceable or readily available potassium, and to which were added increasing amounts of a soluble potassium salt. The results of these tests are given in table 1. The data show that at any one level of potassium the different strains of Aspergilli

TABLE 1

Comparison of the weights of mycelium produced by different cultures of Aspergilli

CULTURE	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES			
	K_2O added per 100 gm of soil			
	10 mgm. K_2O	20 mgm. K_2O	30 mgm. K_2O	40 mgm. K_2O
	gm.	*gm.*	*gm.*	*gm.*
A. niger 1	0 96	2.29	3 51	3.81
A. niger 2	0.92	1.68	3.30	4.33
A. niger 3	1 31	2.20	3.10	3 90
A. niger 4	1 43	3.05	3 86	4 05
A. niger 5	1 08	2.56	3 50	4 45
A. niger 6	1.58	4.20	3.56	3.78
A. niger 7	1.70	3.60	3.90	3.55
A. fischeri 5041	1 63	2 36	2.89	3.64

produce different weights of mycelium. Strains 1, 2, and 5 seemed to be the most promising from the standpoint of weight in relation to available potassium. Strain 1 is an old stock culture of the department of bacteriology; strains 2, 3, 6, and 7 are recent isolations; and strain 5 comes from the Weihenstephan Station, Germany, and was obtained through the courtesy of Dr. Niklas and Dr. Poschenrieder. *A. fischeri* 5041 was a culture originally obtained from the U. S. Bureau of Chemistry and Soils, and now maintained in the stock collection at this station. The results of these tests clearly indicate the superiority of strain 5, and hence this culture was selected for use in all further experiments. Strain 4 formed only a fragile pad and thus was difficult to handle. Strain 2 closely resembled strain 5, and for this reason was used in tests of a number of soils, but it was found that the total weight of mycelium from four flasks seldom was more than 1.70 gm., whereas strain 5 proved more vigorous. Strains 6 and 7 produced maximum yields at the lower concentrations of potassium and hence were unsuitable.

Effect of age of spores on growth of fungus

To maintain physiological stability of stock cultures, the medium of Trischler (24), or a medium used here has been found satisfactory. The latter medium consists of 25 gm. malt extract, 20 gm. agar, and 1,000 cc. of water. Slant cultures on this medium are incubated at 30° to 35°C. until complete conidial formation takes place and then placed in an ice box. Stock cultures may be kept on this medium for at least 4 months without any loss in activity.

From these stock cultures inoculation is made to a medium low in phosphorus as proposed by Trischler (24) or to slants of the malt extract agar medium just described. After about 10 days' incubation at room temperature they are ready for use. The spores are washed out with two 5-cc. portions of water after they have been brushed loose with a wire loop. Of this suspension 0.5 cc. is used to inoculate each soil culture flask.

In order to determine the relation between age of spores and the weight of pad produced, a series of tests were made with spores from cultures kept at room temperature for 20, 40, and 60 days. The results of these tests showed that within the age limit of 20 to 60 days, *A. niger*, strain 5, produced approximately the same weight of pad. Somewhat similar findings have been reported by Frey and Poschenrieder (4), who carried on an extended study of this phase. They found that the highest weights of mycelium are obtained when spores from cultures 1 to 3.5 months old are used, and that older cultures give inconsistent yields, especially with media of low and very high potassium contents. It may be concluded that spore suspensions prepared from cultures not more than 60 days old will give the best results.

Factors influencing the weight of mycelium

Influence of reaction. In order to prevent the interference arising from the growth of various kinds of soil microörganisms, it is necessary either to sterilize the soil or else to provide a reaction which is sufficiently acid to prevent this growth and still allow *A. niger* to make a vigorous growth. Sterilization has not been found very satisfactory. The influence of the reaction on the weight of *A. niger* has been studied repeatedly in solution cultures under controlled conditions. In this investigation it was important to study the influence of reaction on the weight of mycelium when soil was present in the culture. The optimum reaction for conidia germination is pH 3.1 (9), and for growth it varies, the maximum weight of mycelium under sterile conditions being obtained when the acids produced are neutralized by the presence of an excess of $CaCO_3$. However, the increase in weight for *A. niger* due to the $CaCO_3$ is not as marked as for other species of *Aspergilli.* Niklas recommends 1.0 per cent citric acid in the medium giving pH 2.4 for the medium alone, and pH 2.4 to 2.8 in the presence of soils containing less than 1 per cent $CaCO_3$. In the presence of soils having more than 1 per cent of $CaCO_3$, the initial reaction is less acid and such soils should be neutralized with additional citric acid, thus maintaining a pH below the growth stage of most soil bacteria.

It may be well to describe a simple method for determining the carbonate content of soils, so that provision may be made for the addition of sufficient acid. Only soils which effervesce need to be neutralized. The method depends upon the evolution and volumetric measurement of the CO_2 evolved when the soil is treated with an acid. The apparatus consists of two 50-cc. Mohr burettes connected at the lower ends with about 2 feet of rubber tubing, and about one-half filled with CO_2-saturated water. The upper end of one burette is left open and to the upper end of the other is connected a 60 to 100-cc. wide-mouthed bottle. This bottle is provided with a one-hole rubber stopper carrying a glass tube having an elongated bulb at the lower end, of about 10 cc. capacity, which projects into the bottle. The glass bulb is provided with an opening about midway up on one side. The upper end of the tube is connected with the burette by means of rubber tubing.

The method of operation is as follows: One gram of soil is placed in the bottle (if carbonate content is very high, use 0.5 gm.) and 3 to 4 cc. of 5*N* HCl is placed in the bulb. The stopper is then tightly inserted in the bottle and the upper end of the tube connected to the burette. After the height of the liquid in the burette has been read, the bottle is tipped, causing the acid to flow out of the bulb onto the soil. The level of the liquid in the two burettes is kept about the same so as to avoid pressure and absorption of evolved CO_2, and when evolution of CO_2 is complete, the liquid in the two burettes is brought to exactly the same level and the burette is read again and the increase in volume of gas noted. The number of cubic centimeters of CO_2 evolved, times a factor, which, for a 1-gm. sample, is 0.405 at 25°C. and 745 mm. pressure, gives directly the percentage of $CaCO_3$ in the soil, and reference to the table by Niklas et al. (10, 14) shows directly how much acid is required to neutralize the carbonate present in the soil.

By reducing the citric acid content of the medium to 0.5 and 0.25 per cent, the weight of *A. niger* is not markedly influenced. With some soils, e.g., 39, 44 and 46 given in table 2, 0.25 per cent citric acid did not entirely suppress the natural microflora of the soil, and consequently low weights of mycelium were obtained. Tests indicated that when soil is not added and the citric acid is reduced to 0.5 per cent, the weight of mycelium produced will increase somewhat over that produced with 1 per cent of citric acid. Thus for germination and initial growth a reduced total acidity is beneficial. In the presence of soil, a portion of the acid is neutralized by the soil constituents, bringing about an optimum level. A reduction of citric acid to 0.5 per cent may be permissible with acid soils, but the improvement is negligible.

Sterilization and partial sterilization experiments were carried out which served mainly to indicate that equally reliable results can be obtained by the addition of citric acid with less labor and time. Mold spores can usually be killed in 5 minutes at 60°C. under moist conditions (29). Such heat treatments were made on several soils and no mold growth developed after 4 days' incubation. If, however, the initial reaction of the substrata is above pH 5.0, partial sterilization will stimulate bacteria, mainly the rod shaped spore-

formers, to such an extent as to limit or even make impossible the germination of the *A. niger* spores.

In the utilization of $(NH_4)_2SO_4$ by *A. niger*, sulfuric acid is liberated, and the greater the supply of potassium in the medium the greater the mold growth and the greater is this liberation. This results in a lowering of the pH which, however, is not serious as regards the results obtained. There is a voluminous literature in regard to nitrogen sources for *A. niger*, which, in the main, emphasizes the fact that the best sources are ammonium salts (19) and of these ammonium sulfate appears best.

TABLE 2

The influence of strength of citric acid in culture on the weights of mycelium of A. niger when grown on different soils

SOIL NUMBER	REPLACEABLE K_2O PER 100 GM. OF SOIL	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES		
		0.25 per cent citric acid in culture	0 5 per cent citric acid in culture	1.0 per cent citric acid in culture
	mgm.	*gm.*	*gm.*	*gm.*
1	0.7	0.79	0.94	0.92
4	3.5	0.91	0.98	0.98
8	5.5	1.10	1.06	1.02
15	7.2	1.29	1.16	1.22
17	7.6	1.21	1 16	1.00
20	8 3	1.40	1.46	1.37*
34	11 2	1.25	1.28	1.33
37	12.6	1 46	1.30	1.34
39	13.1	1.07	1.38	1.54
44	14.2	1 13	1 63	1.40
45	14 7	1.81	1 93	1.88
46	15.0	1 65	2 07	2 24
51	16.7	1.25	1 80	1.77
53	19.2	1 58	1.66	2.00
58	20 9	1 77	1 94	1 80
60	22 6	1 56	1 77	1 78

Influence of various salts. Other factors influencing the weight of mycelium are time and temperature of incubation, amount of spore suspension used as inoculum, and concentration of nutrients. Most of these factors can be standardized and do not cause serious errors in the results. As a result of the great variations of soil type, however, changing nutritive influences are to be expected. Thus Vageler and Alten (25) found that Ca, Mg, and Na salts stimulate the growth of *A. niger* and give high potassium values for alkaline soils. Similar observations and studies were made by Vilsmeier (27) and Niklas, Vilsmeier, and Poschenrieder (17). Kiessling (6) also found that humic acid increases the weight of mycelium.

The influence of Ca, Mg, and Na carbonates on the growth of *A. niger* in soil culture was studied. Two soils which are on the borderline as regards the

need of potash fertilizer were selected and treated with increasing amounts of Ca, Mg, and Na carbonates in different combinations. The results showed that there is no increase in the weight of the mycelium but rather a decrease with increased additions of Ca, Mg, and Na carbonates. Tests with different Ca compounds and different amounts when added to soil cultures did not show an increase in the weight of the mycelium as compared to the non-treated cultures. From table 3 it is evident that the presence of $CaCO_3$ in soils does not materially increase the weight of mycelium, and the correlation with the Neubauer values is apparently not influenced by the $CaCO_3$ content. The results do not support the need of the correction for calcareous soils suggested by Niklas and Poschenrieder (10).

TABLE 3

The influence of the $CaCO_3$ content of soils on the weights of mycelium of A. niger as compared to Neubauer values on these soils

SOIL NUMBER	$CaCO_3$ CONTENT OF SOIL	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	K_2O PER 100 GM. SOIL		TOTAL SOLUBLE SALTS
			A. niger method	Neubauer method	
	per cent	*gm.*	*mgm.*	*mgm.*	*p.p.m.*
1649	36.4	0 87	8 6	0.6	1115
1800	0.0	0 84	8 0	1.2	165
1646	3 0	1.18	12 4	3 6	413
1798	0 0	1.09	11 3	4 8	82
1651	29.2	2 13	22 0	18 8	726
1581	0.0	2.10	21 8	18 2	702
1650	38 7	1.72	18 4	13 3	586
1648	0 0	1 77	18.8	13 3	616
1843	2 1	1.16	12 4	5 4	413
1738	0 0	1 46	15.6	5.4	173

It was found that the addition of $CaHPO_4 \cdot 2H_2O$ to the medium acts as a buffer and prevents the culture from becoming too acid. It favors the production of a mycelium with a firm structure. In the subsequent work, 0.072 per cent $CaHPO_4 \cdot 2H_2O$ was added to the nutrient solution.

Influence of acid radicals of potassium salts. Trischler (24) indicated that the addition of chemically equivalent amounts of different potassium salts gave different weights of mycelium, and concludes that *A. niger* cannot be used for accurate quantitative measurement of available potassium. This point was investigated, and the results are given in table 4. It can be seen that the differences in weight of mycelium are not very great, with the possible exception that when K_3PO_4 was used somewhat low results were obtained.

Influence of other soil constituents and stimulants. Steinberg (23) reported that many salts, including those of the heavy metals, stimulate the growth of *A. niger*. Since such constituents may be found in certain soils, it seemed desirable to study the effect of them. A fertile soil and soil extracts from it

were added to cultures containing the regular culture medium, but varying amounts of soluble potash. At the higher concentrations of potash, where nearly maximum growth took place, the addition of soil or soil extract caused a slight improvement in growth of *A. niger* and prevented the usual decrease in growth that ordinarily takes place with excessive concentrations of soluble potash. It may be concluded that any effect brought about by certain soils, because of their content of special constituents and stimulants, is insufficient to influence the results of the method for practical purposes.

TABLE 4

The effect of adding different forms of potassium salts on the weights of mycelium of A. niger

K_2O added	Forms of potassium salts added and total weights of mycelium from 4 cultures					
	KCl	K_2CO_3	K_2SO_4	KNO_3	K_3PO_4	KH_2PO_4
per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.001	1 64	1.83	1 61	1.75	1 48	1.69
0.002	2.39	2.51	2 04	2.27	2 05	2.17
0.003	2.85	2 95	2.64	2.66	2.41	2 54
0.004	3.68	3.88	3.07	3 23	2.66	3.07
0.005	4.35	4.42	3.88	3.94	3 58	3.89
0.006	4.70	4 84	4.31	4 48	4.16	4.01
0.007	4.64	4.76	4.58	4.75	4 40	4.67
0.008	4 60	4.55	4.74	4.77	4.72	4.93

Description of method finally adopted

A nutrient medium is made up according to the method of Niklas et al. (see p. 260), excepting that 0.72 gm. of $CaHPO_4 \cdot 2H_2O$ per liter is added, which acts as a buffer, furnishes calcium, and was found to help in the production of a firmer and better pad, especially with the very acid soils. If the medium is to be stored more than 2 days before use, it should be sterilized at about 115°C. for 5 minutes.

The soil sample is air dried, passed through a 20-mesh sieve, and 2.5-gm. portions in triplicate or quadruplicate are placed in 125-cc. Erlenmeyer flasks, which have been thoroughly washed but not sterilized. In the case of peats, the sample is wrapped in potash-free filter paper and then dropped into the flask. Otherwise it is difficult, later, to separate the mycelium from the peat, which tends to float. Nutrient medium, 30 cc. per flask, and then spore suspension, 0.5 cc. per flask, are added. The flasks are closed with cotton plugs, and the cultures incubated at 35°C. for 4½ days. The appearance of the cultures at this stage is shown in plate 1. An experienced operator can, by casual observation of the size and character of the pads at this stage, determine fairly well whether or not a soil is deficient in potash.

In order to obtain more definite results, the pads are taken out with a forceps, washed with running tap water to remove soil particles and nutrient

medium, pressed in the hand to dispel excess water, placed in evaporating dishes, and dried at 70° to 90°C. for 12 to 14 hours and then at 105°C. for 2 hours. The pads are then placed in a desiccator and later weighed. The total weight of four pads, or of three pads calculated to a four-pad basis, gives, by interpolation on curve II of figure 1, the milligrams of available K_2O per 100 gm. of soil.

If it is desired to analyze the pads for potash, they should be washed with distilled water in place of tap water, and after being dried and weighed in the usual way the pads are ground in a mortar. The pulverized tissue from two or three cultures is placed in a 50-cc. porcelain crucible and moistened thoroughly with a saturated alcoholic solution of magnesium nitrate. The alcohol is largely driven off with a low flame, and then the pads are finally ignited at a low temperature just high enough to give a slight redness to the bottom of the crucibles. It is very important that the temperature in the crucible does not exceed this; otherwise there may be loss of potash. The ash is crushed and then extracted with about 200 cc. of hot water on a filter paper, and the potash determined in the filtrate.

Application of method to the quantitative determination of available potassium

Not until recently has Niklas (10) suggested a method of evaluating the weights of *A. niger*, whereby the need of potash fertilization is indicated when the weights fall below certain limits. It seemed very desirable to develop a procedure which permits a quantitative determination. For this purpose the pads produced were analyzed for potassium and the results calculated on a basis of the amount of K_2O which would be absorbed from 100 gm. of soil, so that comparison might be made with other methods.

Analysis of the mycelium for potassium. Seventy-four soil samples were obtained from various sections of the United States, Hawaii, and East Prussia, Germany, and tested by the *A. niger* method for available potassium, and by a chemical method for replaceable potassium. The results of this work are given in table 6. It will be noted that *A. niger* absorbed more potash than is represented by the replaceable potash. This is probably because a mold, in growing for a period of 5 days, is able to utilize an appreciable amount of potash going into solution slowly from the feldspars and micas, whereas the method for replaceable potash, involving an extraction for a 5-minute period only, removes little other than replaceable potash. In all other respects the correlation between the two methods is quite uniform, and after once the results of the *A. niger* method are standardized with soils of known potash needs, the method can undoubtedly be used quite satisfactorily for determining the potash requirements of soils. In order to simplify the method for practical purposes, so as to eliminate the need of making potash determinations on the mycelium, it was decided to attempt to get the approximate amounts of potash absorbed by means of an interpolation method.

Interpolation on a curve. In order to determine the weights of the mycelium which would be produced with different amounts of soluble potash, *A. niger* was grown in regular cultures to which were added varying amounts of KNO_3 and soil extract with a little soil. The results of this experiment are given in table 5. Using the weights of soluble potash present in the medium as abscissae, and the weights of mycelium produced as ordinates, curve I in figure 1 was drawn. The data obtained with 74 soils, given in table 6, are also expressed in figure 1. Each cross represents the point of intersection between

TABLE 5

The weights of mycelium produced by soil cultures to which KNO_3 and soil extract were added

K_2O added as salt or soil extract on the basis of 100 gm. of soil	Total weights of mycelium after 5 days from 4 cultures	
	When soil extract was added	When KNO_3 was added
mgm.	*gm.*	*gm.*
2	0.78	0 82
4	0.99	1 03
6	1.15	1.21
8	1.34	1.36
10	1.62	1.58
12	1.83	1.77
14	1.94	1.98
16	2.13	2.13
18	2.33	2.35
20	2.44	2.46
22	2.63	2.65
24	2.80	2.76
28	3.07	3 13
32	3.39	3.37
36	3.50	3.62
40	3.82	3.90
50	4.20	4.38
60	4.59	4.61
70	4.80	4 76
80	4.79	4.81

the weight of mycelium and potash content of *A. niger* grown on a particular soil. Curve II, drawn midway between these crosses, shows that some of the latter fall a considerable distance from the curve, especially when high amounts of potash are present. This, however, is not a serious objection because the main object of a test of this kind is to distinguish the group of soils low in available potash from the group high in available potash. Since curve II represents data from actual soils, it was used, rather than curve I, in the subsequent work for estimating by interpolation the amount of potash absorbed and available.

TABLE 6

Weights, and potash contents of the mycelium of A. niger grown on 74 different soils, and the replaceable potash of the soils

SOIL NUMBER	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	PERCENTAGES OF K_2O IN MYCELIUM BY ANALYSIS	K_2O ABSORBED BY A. NIGER FROM 100 GM. SOIL	REPLACEABLE K_2O IN 100 GM. SOIL
	gm.	*per cent*	*mgm.*	*mgm.*
1	0.73	0 078	5 60	0 7
1649	0.98	0.071	6.90	7.2
2	0.66	0.109	7.20	1.1
3	0.83	0.096	8 00	2 0
4	0.70	0.114	8 00	3.5
8	0.98	0.082	8 00	5 5
10	0 83	0.096	8.00	5.8
6	0.74	0.143	8.40	4 7
7	0 92	0 091	8.40	5 1
9	0 91	0.092	8 40	5 5
14	0.90	0.093	8 40	7.0
19	0.94	0 097	9.10	7 8
31	1.16	0 080	9.30	9.9
5	0.98	0.097	9 50	4 6
17	0.82	0 125	10 20	7.6
16	1 00	0 105	10.50	7 3
21	1.04	0 103	10 70	8 3
15	1 24	0 087	10 80	7 2
1801	0.85	0.137	10.90	2.1
34	1.15	0 100	11 50	11.2
1646	1.18	0.102	12 01	8.9
28	1.33	0 105	14 10	9.4
37	1.43	0.102	14.60	12.6
1754	1 16	0 133	14.66	7.7
1634	1 80	0 088	15 18	8 8
46	1 83	0 086	15 70	15.0
77	2 03	0 083	16 60	10.9
50	1 59	0 105	16 70	15.7
1637	1.42	0 120	16 75	9.5
1632	1 57	0 108	16 75	8.7
1798	1 09	0 160	17.27	6.1
1845	1 49	0 116	17.27	9.8
1638	1.48	0 096	17.79	8 5
57	1 60	0 116	18 50	20.7
1845	1 49	0.127	18.84	9.8
1740	2 01	0 093	18.84	13.4
59	1.85	0 103	19 00	21.4
1648	1.77	0 108	19.10	14.3
73	1 89	0 102	19 36	13.15
1799	1.10	0.179	19 36	5.0
1581	2.10	0.101	21.04	18.5
1739	2.76	0.082	22.51	23.2
1740	2.01	0.120	23.55	13.4
1633	1.64	0 145	23.66	13.6
1577	1.92	0 128	24 35	14.4

TABLE 6—*Concluded*

SOIL NUMBER	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	PERCENTAGES OF K_2O IN MYCELIUM BY ANALYSIS	K_2O ABSORBED BY A. NIGER FROM 100 GM. SOIL	REPLACEABLE K_2O IN 100 GM. SOIL
	gm.	*per cent*	*mgm.*	*mgm.*
80	2.80	0.089	25.12	21.6
1643	1.69	0.161	27.20	10.9
79	3.50	0.092	32.36	38.2
1578	2.84	0.116	32.45	29.7
1844	3.05	0.110	33.50	17.0
72	4.28	0.079	34.02	26.0
67	4.42	0.082	36.11	27.8
62	3.50	0.104	36.30	41.9
1755	4.50	0.081	36.64	34.8
71	4.90	0.075	36.64	30.6
63	3.85	0.098	37.69	19.2
68	3.97	0.095	37.70	21.5
69	3.95	0.097	38 48	19.5
70	4 35	0.090	39.26	28.5
66	4.48	0.091	40.57	29.9
82	3.48	0.122	42.67	45.2
78	3.44	0.126	43.45	38.8
1848	4.61	0.100	46.32	37.9
75	4.55	0.104	47.11	29.5
74	4.90	0.097	48.16	29.3
1595	4.10	0.121	50.08	41.9
65	4.84	0.104	50.25	31.4
64	4.89	0.104	50.78	40.3
1846	3.84	0.133	50.78	29.4
81	4 60	0.112	51.86	46.1
1850	4.00	0.138	54.96	26.3
1590	4.54	0.130	59.69	59.1
1755	4.50	0.148	65.97	34.8
1582	3.97	0.087	68.30	108.2

Comparison of results with other methods

In tables 7, 8, and 9 the results of analyses with the *A. niger*, chemical, Neubauer, and Mitscherlich methods are given for a large number of soils.

Comparison with a chemical method. It is apparent that, in general *A. niger* absorbs more than the replaceable potassium, excepting in cases where the soil contains an abnormally high content. In these cases there is, apparently, more replaceable potash present than the mold is capable of utilizing. As indicated in figure 2, there exists a fairly good correlation between replaceable potash and that determined by the *A. niger* method.

Comparison with the Neubauer method. A. niger absorbs considerably more potassium than the rye seedlings in the Neubauer method, especially when the available potassium content is low. High soluble salt content of the soil tends to give somewhat higher values with *A. niger*. Similarly, with peat and muck soils, the *A. niger* method gives slightly higher results, but usually not

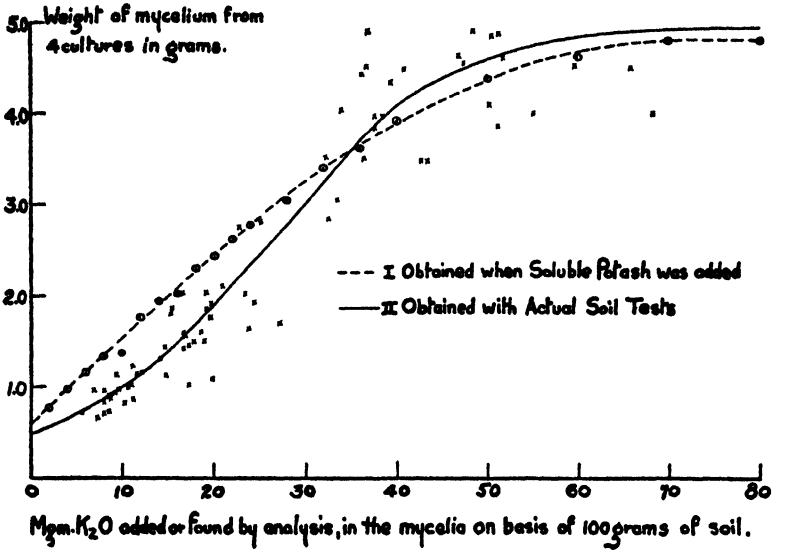

FIG. 1. RELATION OF WEIGHTS OF MYCELIUM TO SOLUBLE POTASH ADDED IN NUTRIENT MEDIA (CURVE I); RELATION OF WEIGHTS OF MYCELIUM TO POTASH EXTRACTED FROM DIFFERENT SOILS BY THE MOLD (CURVE II)

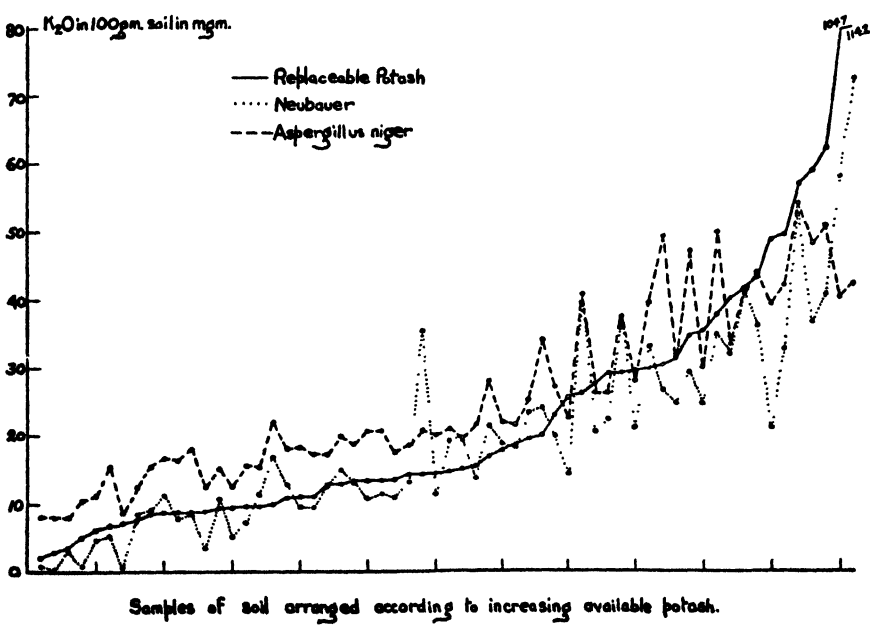

FIG. 2. COMPARISON OF AVAILABLE POTASH BY THREE METHODS IN 61 SAMPLES OF SOIL FROM VARIOUS PARTS OF UNITED STATES

in excess of the replaceable potassium. As shown in figure 2, there is a rather good correlation between the Neubauer and *A. niger* methods.

TABLE 7

Available potash in 62 soils determined with the A. niger and chemical methods

(Curve II, figure 1 used in *A. niger* method)

SOIL NUMBER	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	AVAILABLE K_2O PER 100 GM. SOIL		SOIL NUMBER	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	AVAILABLE K_2O PER 100 GM. SOIL	
		A. niger method	Chemical method (replaceable)			*A. niger* method	Chemical method (replaceable)
	gm.	*mgm.*	*mgm.*		*gm.*	*mgm.*	*mgm.*
1	0.71	6 0	0.7	32	1.38	14.8	10.8
2	0.73	6.1	1.1	33	1.35	14.5	10.8
3	0.86	8.2	2.0	34	1.30	14.0	11 2
4	0.80	7.5	3.5	35	1.40	15.1	11.8
5	0.91	9 0	4.6	36	1 45	15.6	12 0
6	0.89	8 8	4 7	37	1.50	16 1	12.6
7	0.94	9.4	5.1	38	1.50	16 1	12.9
8	0 97	9.8	5.5	39	1.61	17.1	13.1
9	0.94	9 4	5.5	40	1.58	16 8	13.6
10	1 02	10 4	5 8	41	1.54	16.4	13.8
11	1.23	13.2	6.1	42	1.63	17.2	14.0
12	1 18	12.6	6.3	43	1.66	17.8	14.1
13	1.22	11.0	6.5	44	1.88	19.8	14 2
14	1.12	11 8	7.0	45	1.91	20.0	14.7
15	1.08	11.4	7.2	46	1.80	19.1	15.0
16	1.15	12.2	7.2	47	1.75	18.6	15.2
17	1.24	13.3	7.6	48	1.78	18.8	15.5
18	1.26	13.6	7.7	49	1.93	20.2	15.5
19	1.36	14 6	7 8	50	1.88	19 8	15.7
20	1.34	14 4	8 3	51	2.00	20.9	16.7
21	1 33	14 3	8.3	52	1.85	19.6	19.0
22	1 16	12 3	8.3	53	1.73	18.5	19.2
23	1.20	12.8	8.7	54	1.95	20.3	19.5
24	1.34	14.4	8 8	55	2.12	22.0	20.0
25	1.23	13.2	8 8	56	2.14	22.2	20.1
26	1.48	15.9	8 9	57	2.23	22.9	20.7
27	1.41	15.2	8.95	58	2.17	22.4	20.9
28	1.35	14.5	9.4	59	2.27	23.2	21.4
29	1.34	14.4	9.7	60	2.34	23.8	22.6
30	1 40	15.1	9.8	61	2.54	25.6	26.1
31	1 39	14.9	9.95	62	3.52	34.4	41.9

Comparison with the Mitscherlich method. The Mitscherlich method gives lower values for available potash than the *A. niger* method. However, in the soils tested, both methods agree in that none of the soils need potash fertilization

TABLE 8

Available potash in 61 soils according to the A. niger, Neubauer, and chemical methods

(Curve II, figure 1 used in *A. niger* method)

SOIL NUMBER	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	AVAILABLE K_2O PER 100 GM. SOIL			SOLUBLE SALT CONTENT OF SOIL
		A. niger method	Neubauer method	Chemical method (replaceable)	
	gm.	*mgm.*	*mgm.*	*mgm.*	*p.p.m.*
1801M*	0 85	8.1	1.8	2.1	111
1800	0.84	8 0	1.2	2 9	165
83	0.85	8.0	3.1	3 5	....
1799	1 10	10.4	1.8	5 0	70
1798	1.09	11.3	4 8	6.1	82
1738	1.46	15 6	5 4	6 9	173
1649M	0 87	8.6	0.6	7 2	1115
1754	1.16	12.4	8 5	7 7	181
1638	1 48	15.7	9.1	8 5	235
1632	1 57	16.8	11 5	8.7	151
1635	1 54	16 4	7 9	8 8	192
1634	1.70	18 0	8 5	8.8	270
1646M	1 18	12 4	3 6	8 9	413
1637	1 42	15 2	10 9	9 5	221
1843	1.16	12 4	5 4	9 6	413
1845	1 49	15 8	7.3	9 8	199
1645	1 46	15 6	11 5	9 8	197
1641	2.13	22 0	16 9	10 0	200
1643	1.69	18 0	12.7	10 9	224
1631	1.71	18.2	9.7	11.1	156
1647	1 62	17 3	9 7	11 2	413
1644	1 61	17.2	12.7	12.8	154
1640	1 88	20.0	15.1	12 8	275
1648M	1 77	18 8	13 3	13 4	616
1639	2 00	20 7	10 9	13 4	181
1740	2 01	20 8	11 5	13 4	140
1633M	1 64	17 6	9 1	13 6	200
1650M	1 72	18 4	13 3	14 3	586
1847	2.00	20 8	35 7	14 3	1183
1577M	1 92	20 2	11 5	14 4	1234
1642	2 02	21.0	19 4	14.7	419
84	1 87	19.6	19.8	15 0	...
1636	2 09	21 8	13 9	15 7	192
1844	2.86	28 2	21 8	17.0	346
1651M	2 13	22 0	18 8	17.9	726
1581M	2 10	21.8	18.2	18.5	702
1737	2 52	25 4	23.6	19 8	273
1756M	3 52	34.2	24.2	20.0	306
1739	2 76	27 4	20 0	23.2	275
1593M	2.19	22 6	14 5	25.9	3046
1850	4.00	39.8	41.1	26 3	381
1489P†	2.65	26.5	20 6	27.8	3510

* M = Muck.

† P = Peat.

TABLE 8—*Concluded*

SOIL NUMBER	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	AVAILABLE K_2O PER 100 GM. SOIL			SOLUBLE SALT CONTENT OF SOIL
		A. niger method	Neubauer method	Chemical method (replaceable)	
	gm.	*mgm.*	*mgm.*	*mgm.*	*p. p. m.*
1585P	2.64	26.4	22.4	29.4	2335
1846	3.84	37.8	36.3	29.4	464
1578M	2.84	28.1	21.2	29.7	3272
1753	4.00	39.8	33.3	30.0	418
1849	4.60	49.6	26.6	30.5	726
1576M	3.22	31.4	24.8	31.4	1847
1755	4.50	47.2	29.6	34.8	200
1588P	3.05	30.0	24.8	35.6	3102
1848	4.61	50.0	35.1	37.9	464
1594P	3.44	33.6	32.1	40.4	1528
1595M	4.10	41.0	41.7	41.9	702
1752	4.32	44.2	36.3	43.6	224
1579P	4.00	39.6	21.2	49.0	3510
1592P	4.20	42.4	32.7	49.8	3272
1580P	4.74	54.4	52.6	57.2	1561
1590P	4.54	48.2	36.9	59.1	2835
1586P	4.65	51.2	41.1	62.4	2335
1582P	4.06	40.4	58.3	104.7	5095
1584P	4.19	42.4	72.6	114.2	3810

TABLE 9

Available potash in 20 soils, determined with the A. niger, chemical, and Mitscherlich methods

SOIL NUMBER	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	AVAILABLE K_2O PER 100 GM. SOIL			
		A. niger method		Chemical method (replaceable)	Mitscherlich method
		By actual mycelium analysis	By curve interpolation		
	gm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
63	3 85	37.69	37.8	19.15	19.64
64	4.89	50.78	62 8	40.25	25.53
65	4.84	50.25	60.0	31.40	98.20
66	4.48	40.57	47.0	29.95	25.53
67	4.42	36.11	46.2	27.85	23.57
68	3.97	37.70	39.2	21.50	23.57
69	3.95	38.48	39.0	19.50	29.46
70	4.35	39.26	44.6	28.50	19.64
71	4.90	36.64	68.0	30.65	25.53
72	4.28	34.02	43.6	26.00	31.42
73	1.89	19.36	20.0	13.15	31.42
74	4.90	48.16	68.0	29.25	24.53
75	4.55	47.11	48.4	29.45	27.50
76	2.72	16.60	22.1	19.70	33.39
77	2.03	16.60	21.2	10.90	29.46
78	3.44	43.45	35.6	38.80	28.00
79	3.50	32.36	34.2	38.20	19.15
80	2.80	25.12	27.8	21.60	19.64
81	4.60	51.86	49.8	46.10	24.06
82	3 48	42.67	34.0	45.20	18.17

Comparison with field tests. In table 10 are given the results of the *A. niger* and chemical methods when used with a group of soils that have been tested in the field for potash needs. The response of alfalfa to potash fertilization on these soils is also given. It will be noted that the lower values in the *A. niger* method, and also in the chemical method, correspond with the soils that respond to potash fertilization.

Practical application

From these data it may be concluded that the need of potash fertilization is indicated when the potash absorbed by *A. niger* is less than 15 mgm. per 100

TABLE 10

Comparison of available potash by A. niger and chemical methods, with response of alfalfa in field to potash fertilization

SOIL NUMBER	TOTAL WEIGHTS OF MYCELIUM FROM 4 CULTURES	AVAILABLE K_2O PER 100 GM. SOIL		INCREASES PER ACRE OF ALFALFA HAY DUE TO POTASH FERTILIZATION
		By *A. niger* method curve interpolation	By chemical method (replaceable)	
	gm.	*mgm.*	*mgm.*	*pounds*
3	0 98	10 0	2 0	408
10	1 32	14.2	5.8	621
12	1.34	14 4	6 3	543
14	1 68	18 0	7 0	193
26	1 72	18 3	8 9	122
28	1.62	17.4	9 4	121
38	1 74	18.4	12 9	98
39	1 94	20.3	13 1	0
43	2.30	21 8	14 1	46
45	2 33	23.6	14 7	65
47	2 45	24 8	15 2	0
51	2 05	21 4	16 7	0
54	2 51	25 3	19 5	0
61	2 81	27.8	26 1	0

gm. of soil. If the amount is 15 to 20 mgm., a light potash application may be beneficial, and when it is more than 20 mgm., there is probably no need for potash fertilization, excepting for certain special crops and conditions.

SUMMARY

In a study of the *A. niger* method for the determination of available potassium in soils, attention was given to the following points: (*a*) adaptability of various strains of *A. niger;* (*b*) maintenance of stock cultures; (*c*) influence of reaction, various salts, acid radicals and minor soil constituents and stimulants; (*d*) application of the method to the quantitative determination of available potassium by means of analysis of the mycelium for potassium, and by interpolation on a specially constructed curve; and (*e*) comparison of results

with other methods and field tests. The results of this study may be summarized as follows:

Two strains of *A. niger*, one obtained from Weihenstephan, Germany, and which has been used by Niklas, and one from the stock cultures at this station gave equally satisfactory results. The strain from Germany was chosen for the work reported here because of its already wide use in this connection by Niklas and others.

It was found that when the $CaCO_3$ content of the soil exceeded 1 per cent it is necessary to add additional acid to the cultures in order to maintain an optimum pH for the germination of the mold spores and to prevent bacterial growth. A simple method for determining the $CaCO_3$ content of the soil is described.

It was found that various Ca, Mg, and Na salts, acid radicals of potassium salts, and minor soil constituents and stimulants do not influence the growth of the mold sufficiently to interfere seriously with the results.

The method can be applied to the quantitative determination of available potassium by means of analysis of the mycelium for potassium or more conveniently by interpolation of the weight of the mycelium on a curve which has been especially constructed for this purpose.

The results with the method compare very favorably with the chemical method, the Neubauer method, Mitscherlich's method, and field tests.

It is concluded that the test is simple and reliable and may be used in a practical way for the determination of potash needs of soils.

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PLATE 1

GROWTH OF A. NIGER CULTURE WITH DIFFERENT SOILS

1—Plainfield sand—contains 0.7 mgm. replaceable K_2O per 100 gm. soil. Needs potash.

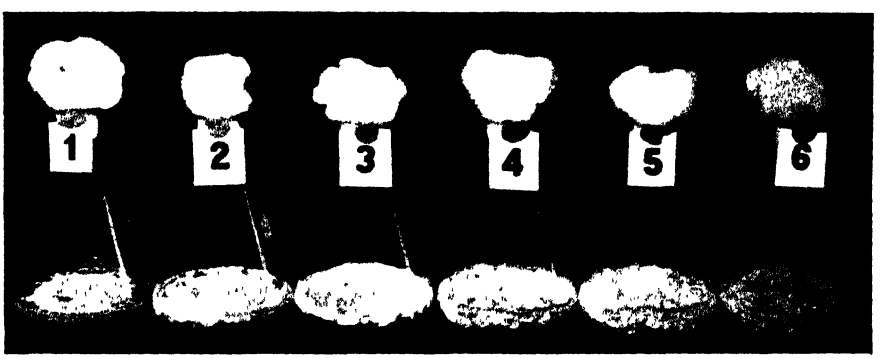
2—Sandy loam—contains 5.8 mgm. replaceable K_2O per 100 gm. of soil. Needs potash.

3—Marshall silt loam—contains 11.8 mgm. replaceable K_2O per 100 gm. of soil. Slight need of potash.

4—Silt loam—contains 19.8 mgm. replaceable K_2O per 100 gm. of soil. Does not need potash.

5—Silt loam—contains 37.9 mgm. replaceable K_2O per 100 gm. of soil. Does not need potash.

6—Soil from Prof. Mitscherlich, Germany—contains 40.25 mgm. replaceable K_2O per 100 gm. soil. Does not need potash.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PHOTONITRIFICATION IN SOIL

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In previous publications[1] from these laboratories a new view on the process of nitrification in soil has been advanced and supported by experiments. It has been shown that the process of nitrification in soil is mainly due to the oxidation of ammonium salts to nitrite by air in the presence of sunlight. In other words, according to our view, nitrification in the soil, especially in the tropics, is more photochemical in nature than bacterial.

In this paper, we are submitting the results of our experiments on nitrification in soil effected by sunlight. The experiments were carried on as follows:

Soil was collected from a hole dug 9 inches deep in the grass land at a particular spot in the laboratory compound. The soil was crushed, passed through a sieve with 1 mm. bore, and was air dried at 30°. Of this soil 2,000 gm. was mixed with 20 gm. of ammonium salts and 750 cc. of water. The whole mixture was kept in both earthen and large glass jars having a capacity of 4,000 cc. and exposed to sunlight. Water was daily added in order to make up for the water lost by evaporation. The vessels were weighed every week to test for the constancy in weight. The soil was well stirred every morning before exposure. Blank experiments were also carried on in the dark by covering the glass jars with a thick layer of black Japan enamel and the earthen pots with a tin lid blackened by black Japan. For the experiments in the dark, these vessels were also placed in the sun to have the same temperature as those exposed to the sun. After the exposure was completed 50 gm. of the soil was taken out and the ammonia was estimated by adding potassium hydroxide to the soil and distilling the liberated ammonia, which was absorbed by a standard solution of sulfuric acid. At the end of the experiment, this sulfuric acid was titrated against the standard solution of caustic potash and thus the amount of ammonia absorbed by sulfuric acid was found. Then another 50 gm. of the soil was taken and treated with Devarda's alloy for estimating the ammonium salt and the nitrites and nitrates.

Table 1 shows the results obtained with soil and ammonium salts exposed in earthen vessels for 160 hours. These experiments carried on in earthen

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pots were not very satisfactory because the pots were porous and, as a result, a good deal of the ammonium salts percolated and came out on the outer surface of the pots.

The results given in table 2 were obtained in glass vessels after 160 hours' exposure to sunlight with loose covers. As the percentage of oxidation was

TABLE 1

Nitrification of soil exposed in earthen vessels for 160 hours

NUMBER	CONDITION	AMOUNT OF SALT ADDED	UNCHANGED AMOUNT OF SALT	AMOUNT OF OXIDIZED SALT	PERCENTAGE OXIDIZED
1	Sunlight	20 gm. ammonium chloride	13 gm. ammonium chloride	6.7 gm. ammonium chloride	33.5
2	Dark	20 gm. ammonium chloride	18.7 gm. ammonium chloride	1.3 gm. ammonium chloride	6.5
3	Sunlight	20 gm. ammonium chloride to heated soil	15.5 gm. ammonium chloride	4.4 gm. ammonium chloride	22 0
4	Sunlight	20 gm. ammonium sulfate	18.75 gm. ammonium sulfate	1.2 gm. ammonium sulfate	'6 0
5	Dark	20 gm. ammonium sulfate	20 gm. ammonium sulfate	nil	nil
6	Sunlight	20 gm. ammonium phosphate	16.6 gm. ammonium phosphate	3.3 gm. ammonium phosphate	16.5
7	Dark	20 gm. ammonium phosphate	20 gm. ammonium phosphate	nil	nil

TABLE 2

Nitrification of soil exposed in glass vessels for 160 hours

NUMBER	CONDITION	AMOUNT OF SALT ADDED	UNCHANGED AMOUNT OF SALT	AMOUNT OF OXIDIZED SALT	PERCENTAGE OXIDIZED
1	Sunlight	20 gm. ammonium chloride	18.64 gm. ammonium chloride	1.33 gm. ammonium chloride	6 65
2	Sunlight	20 gm ammonium phosphate	17.54 gm. ammonium phosphate	2.44 gm. ammonium phosphate	12.2
3	Dark	20 gm. ammonium phosphate	19.8 gm. ammonium phosphate	0.2 gm. ammonium phosphate	1.0
4	Sunlight	20 gm. ammonium sulfate	19.6 gm. ammonium sulfate	0.3 gm. ammonium sulfate	1.5
5	Dark	20 gm. ammonium sulfate	20 gm. ammonium sulfate	nil	nil

not high after 160 hours' exposure, the vessels were again exposed to sunlight for a much longer period. The results given in table 3 were obtained after a total exposure to sunlight for 700 hours in the months of May, June, and July, 1932.

The tabulated results show conclusively that on exposing the ammonium salt solutions to light in contact with the soil, an appreciable amount of the salts are photochemically oxidized in air after an exposure for 160 hours but the oxidation is greatly increased after 700 hours' exposure, as shown in table 3. Ammonium phosphate undergoes oxidation to a greater extent than the other ammonium salts under smilar conditions. The oxidation in the vessels kept in the dark under identical conditions is very small and in some cases negligible.

If nitrification is mainly bacterial, as is generally believed, the ammonium salts in the vessels kept in the dark should have undergone appreciable oxidation. But the results show that the dark oxidation is exceedingly small in comparison with the photochemical oxidation. Moreover, even with the

TABLE 3

Nitrification of soils exposed to sunlight for 700 hours

NUMBER	CONDITION	AMOUNT OF SALT ADDED	UNCHANGED AMOUNT OF SALT	AMOUNT OF OXIDIZED SALT	PERCENTAGE OXIDIZED
1	Sunlight	20 gm. ammonium chloride	3.5 gm. ammonium chloride	15.25 gm. ammonium chloride	80.1
2	Dark	20 gm. ammonium chloride	17.25 gm. ammonium chloride	0.85 gm. ammonium chloride	4.8
3	Sunlight	20 gm. ammonium sulfate	17.25 gm. ammonium sulfate	2.62 gm. ammonium sulfate	13.2
4	Dark	20 gm. ammonium sulfate	20 gm. ammonium sulfate	nil	nil
5	Sunlight	20 gm. ammonium phosphate	2 gm. ammonium phosphate	16 5 gm. ammonium phosphate	89.0
6	Dark	20 gm. ammonium phosphate	18.5 gm. ammonium phosphate	0.25 gm. ammonium phosphate	1 4
7	Sunlight	10 gm. ammonium phosphate in 1,000 gm. of heated soil	1.38 gm. ammonium phosphate	8.37 gm. ammonium phosphate	85 8

sterilized (by heating for 48 hours at 150°) soil ammonium phosphate undergoes considerable oxidation in the presence of sunlight. The oxidation of ammonium phosphate in the presence of sunlight is practically the same in the sterilized and in the unsterilized soils. It appears, therefore, that nitrification, especially in tropical countries, is more a photochemical than a bacterial process. It will be of interest to note that the ammonium salts are photochemically oxidized to nitrites and not to nitrates in the soil. It is well known that nitrates are converted into nitrites in light.

We have carried on similar experiments with urea, 10 gm. of which were mixed with 1,000 gm. of the same soil and one glass jar was exposed to sunlight for 540 hours under the same conditions as in the case of the ammonium salts. The following results were obtained: amount of urea added, 10 gm.; amount of

unchanged ammonia, 4.7 gm.; amount of oxidized ammonia, 0.7 gm. Hence the percentage of ammonification is 54 and the percentage of its subsequent oxidation to nitrite is 15.

From our experiments, it appears that ammonification is also markedly photochemical in nature. We have been able to convert several nitrogenous compounds into ammonia by exposing the solutions of these compounds to light. Hence it appears to us that in the soil the ammonification of nitrogenous compounds and the subsequent oxidation of ammonium salts to nitrites are markedly accelerated by light, and the process of soil nitrification is more of photochemical than of bacterial origin.

Further experiments on this line on sterilized and unsterilized soils are in progress.

SUMMARY

On exposing ammonium salt solutions mixed with soil to sunlight in presence of air for 700 hours, the amounts of the ammonium salts oxidized are as follows:

	per cent
Ammonium chloride	80
Ammonium phosphate	89
Ammonium sulfate	13.2

With sterilized soil and ammonium phosphate under identical conditions the percentage oxidation was 85.8.

The amount of oxidation by an exposure of 160 hours was less than in the previous case.

The oxidation in the vessels kept in the dark under identical conditions is very small and in some cases negligible.

It is evident from these and other experimental observations that nitrification in soil, especially in tropical countries, is a more photochemical than a bacterial process.

A SOIL TEMPERATURE INSTALLATION[1]

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The importance of soil temperature as an ecological factor influencing both plant and animal life has come only recently to be recognized. Although there are over 1,000 stations in the United States where meteorological observations are made, and in some cases air temperature records have been kept for over 35 years, it is doubtful whether there are more than 20 localities where soil temperature data have been collected over a period of 10 years.

There are many different methods of taking soil temperatures but one of the most adaptable and accurate is by the use of thermocouples, which are ideally suited for the reading of temperatures in inaccessible places such as in insect bodies, under the bark of trees, and at various depths in the soil. For measuring precipitation, and for taking air temperatures and other meterological data, the government weather stations are equipped with standard apparatus. Soil temperature records from different parts of the country, to be strictly comparable, should likewise be obtained, as far as possible, with a similar technique. There are, of course, many factors entering into the taking of soil temperatures which do not need to be considered in the taking of air temperatures. Slope exposure, for example, is, according to Shreve (3), of great importance at high altitudes and high latitudes but has little importance at sea level at low latitudes. The color, texture, and moisture content of the soil, and the character of the plant cover are among the most important factors to be considered, but these variables afford all the more pressing arguments for the adoption of a standard method. Writing of soil temperatures in the United States, Fitton and Brooks (1) observe, "The scantiness of the material now available emphasizes the need for many additional observations of soil temperatures, which should be made under conditions as uniform as possible."

Many of the records now available are for short periods of time, covering only the growing season of crops being studied or other seasons of the year in which the investigators were interested. Soil records, to be of the greatest value, should be taken over long periods of years. It is with this end in view that the writer has undertaken the installation of a soil temperature apparatus which is designed to give many years of service. For the past 2 years soil tempera-

[1] Montana State College, Agricultural Experiment Station, Paper 11, Journal Series.

[2] The writer wishes to express his indebtedness to the Mountain States Telephone and Telegraph Company for many courtesies extended in connection with this study

tures have been taken at the Montana Agricultural Experiment Station, a modification of the set-up used at Minnesota by the writer (2) being used. This, however, was of too temporary a nature, being designed solely for a short-time investigation.

SOIL TEMPERATURE APPARATUS

The recording portion of the system is located inside a heated one-story wooden building. This consists of a terminal box, a switch board, galvanometer, potentiometer, and a Dewar flask containing ice and water (plate 1). The terminal box, in which all outside wires are soldered to numbered brass studs, is filled with insulating compound. The galvanometer is a Leeds and Northrup No. 2420b. of a sensitivity per millimeter division of 25 microvolts.

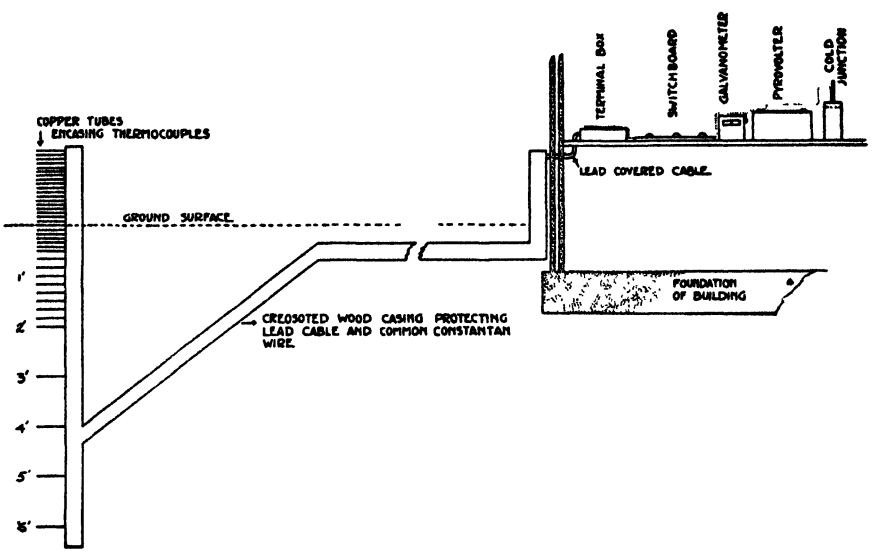

FIG. 1. PLAN SHOWING GENERAL ARRANGEMENT OF SOIL TEMPERATURE INSTALLATION

The potentiometer is a standard Northrup Pyrovolter, catalog No. 160, Range A 0–25°C. 0–1 mv. Range B 0–125°C. 0–5 mv.

The connection from the terminal box to the buried part of the system consists of a lead-covered telephone cable of 52 paper insulated 22-gauge copper wires. The buried portion of the system is 30 feet from the building in a grass lawn. The cable lies in a wooden casing constructed of 1-inch soft pine which, like all the wooden construction, has been treated with two coats of hot creosote. The buried couples are constructed of 32-gauge copper and constantan enameled and single silk insulated wires. All the wiring has been done on a piece of hard wood 1 inch by 4 inches by 6 feet, the thermocouples projecting 6 inches through $\frac{3}{16}$-inch holes bored at the required depths. In order to hold the couples out horizontally from the board and to maintain their relative dis-

tance from each other, as well as to protect them from corrosion, $\frac{3}{16}$-inch copper tubes 7 inches long, sealed at one end and threaded at the other, were slipped over them and screwed into the wood. The wiring being completed and the protecting copper tubes placed in position, the 32-gauge wires were soldered to those of the cable from the recording instruments. This wired hardwood strip was then screwed with flat headed brass screws to a square wooden casing with an inside diameter of 2 inches of which it formed the fourth side. One end being plugged, hot insulating compound, such as is used in transformers, was

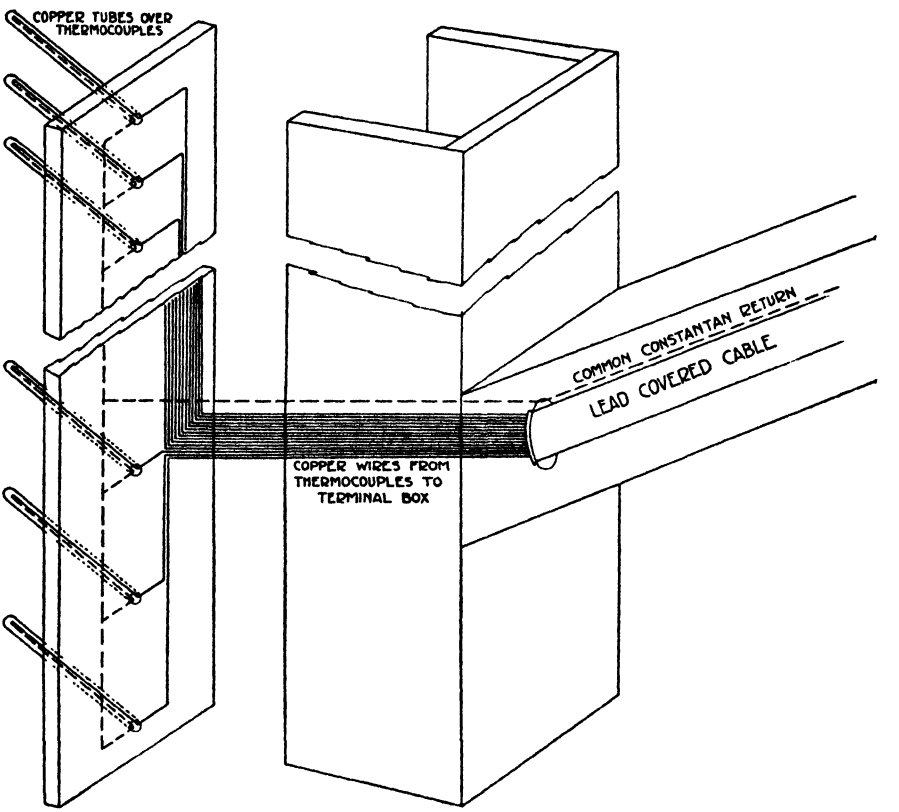

FIG. 2. DETAILS OF CONSTRUCTION INSIDE BURIED WOODEN CASING

poured into the other end thus making the system quite water-tight. There was a chance for error because the lead cable was buried near the surface and might conduct heat to the couples. To obviate this, the cable, about 6 feet from the couples, was inclined downward, and the connections were made with the 32-gauge wires inside their protecting casing at 4 feet in depth. A hole 6 feet deep was dug, with one vertical side. Each foot of earth removed was kept in a separate pile so that it could be returned in the same order in which it was removed. Holes just large enough to receive the copper tubes were bored in

the undisturbed face of the excavation. The wired casing was then placed in position and the hole filled (fig. 1).

The depths at which temperatures are being taken are soil surface, every inch for the first 6 inches, every 2 inches from 8 to 24 inches, and at 3, 4, 5, and 6 feet. There are also couples at inch intervals to a height of 18 inches above ground, the casing extending that distance upward.

The common constantan return wire from the buried thermocouples to the instrument was run with the cable, but not in contact with it, in its protecting wooden casing, no further insulation being considered necessary. The diagram (fig. 2) shows how the system was wired. Where subzero temperatures are likely to be encountered, a double-pole double-throw reversal switch will facilitate making the readings.

A cheaper but satisfactory instrument which may be used in place of the pyrovolter is a Leeds and Northrup Arsonval galvanometer List F4445 Type P with a resistance of 1,100 ohms. This is a wall type with a reflecting mirror and can be used with a lamp and scale or a telescope and scale reading device. In using this instrument in connection with soil temperature work, the galvanometer scale must, of course, be calibrated. This, however, is a simple procedure and, since the temperature scale is a straight line, the readings of deflections can readily be resolved into temperatures. Should the galvanometer scale be of too short a range, a resistance may be introduced which will keep the deflections within measurable range.

Care must be observed in using a galvanometer as part of this installation, as there are sources of error which must be allowed for. Each thermocouple ought to be accurately calibrated, as the resistance of the wires varies according to their length. This is especially important if a low resistance galvanometer is used. Of course, if the variation in the length of the wires is not greater than 5 or 6 feet the error might be so small as to be negligible in this particular work.

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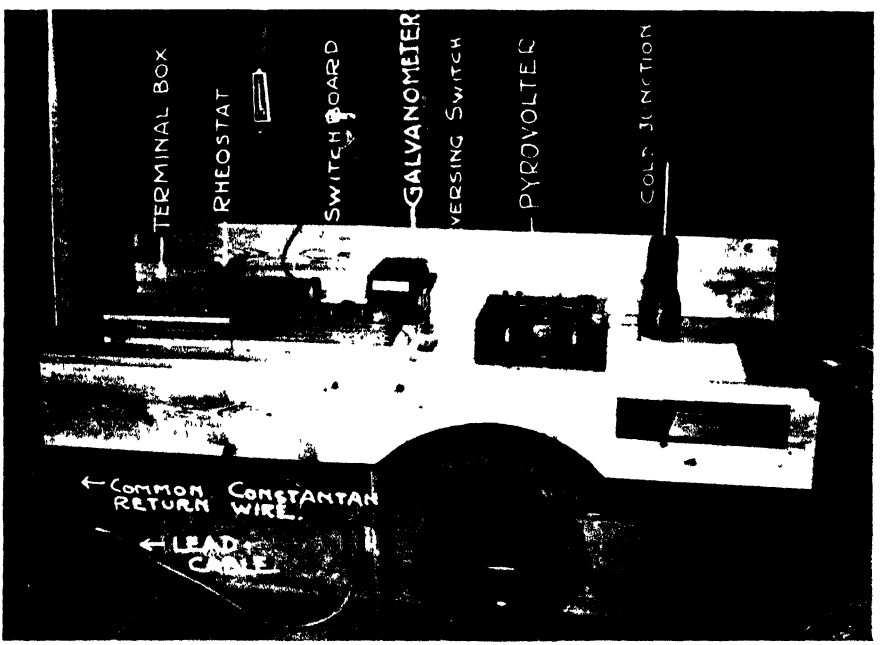

INSTRUMENT BOARD FOR RECORDING SOIL TEMPERATURES

THE DISPERSION OF SOIL-FORMING AGGREGATES[1]

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The primary object in presenting this article is to point out that size distribution is not only dependent on the nature of the soil but also upon the nature of the process used to determine it.

Two arguments are used to support the idea that a unique set of soil particles exist. After a soil has been subjected to a deflocculation treatment, the particles observed under the microscope appear to be unique entities. From this, despite possibilities to the contrary, it is concluded that the essence of the postulate follows. The second is that mechanical analyses derived from standardized processes yield reproducible results. It is generally recognized that all dispersion processes do not accomplish complete deflocculation. Therefore, this argument is not conclusive, for an examination of the literature seems to indicate than an analysis made with any dispersion process is, in a sense, reproducible.

If it were certain that the soil consists of a large number of unambiguously defined particles, and some simple method of treatment might be found to deflocculate the aggregates and place the individual particles in suspension, then it would require nothing essentially new or difficult to make critical analyses of conventional methods. But when the available knowledge of this subject is scrutinized, who is prepared to defend in any satisfactory manner this view?

DUPLICATE SOIL SAMPLES

Soil samples taken from the field at random vary in mechanical composition. For this reason, the analysis is not reproducible unless duplicate samples are employed. Still, it is axiomatic to assume that any given physical system proceeds through a particular set of physical states when it is subjected to an ordered set of definite treatments. If it does not pass through these every time this sequence is applied, failure to reproduce the treatments is regarded as the cause. Soil samples can never be considered more than statistical equivalents. The same thing applies to dispersion processes. Even so, it is expected when a dispersion process is applied to duplicate samples it will pro-

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duce practically the same size distribution of particles, because the definition of *duplicate* predicates this reaction.

The definition of *duplicate* may be deduced from the method of preparing such samples. This is done by mixing the soil and then dividing it into the desired number of portions. The amount of mixing is arbitrary, but it must be enough to make them appear uniform in color and texture. Should they be erratic in reacting to applied processes, the conclusion is that they are not duplicates. In like manner when the problem is reversed, similarity in effect upon the senses and in reaction to processes applied establishes whether or not they are duplicates. Therefore, duplicate samples are those which to an arbitrary degree react alike to similar treatments.

THE DISPERSION OF PARTICLES

The purpose of a dispersion process is to separate the aggregates so that the component particles may settle independently. It is important to emphasize the difficulty of distinguishing between the dispersion of crumbs and the disruption of crystal fragments. From the end products it is not possible to distinguish the two. Nevertheless, mechanical shearing stresses of one kind or another are always employed in dispersion.

In chemical processes experiments fail to show unique dispersion limits. Deflocculation is a continuous function of the concentraton of the reagents applied. Keen and Puri (5) found a different (yet definite) maximum deflocculating ability for a number of reagents. From chemical information it is safe to say that for some soils some deflocculating agents will be associated with values corresponding to solution. This variability of the maximum deflocculating ability seems to indicate that as far as chemical processes of this type are concerned, no unique structure is evident.

In order that there be a unique group of particles, it seems that the forces which hold the crystal fragments together in floccules must be small in comparison with those which act between contiguous parts of them. If the dispersive process breaks some of the fragmentary crystals into smaller pieces at the same time that some of the floccules are being separated into components, there is no way of distinguishing between crystalline fragments derived from floccules and those derived from crystal elements.

IMPERFECTIONS OF CRYSTALS

Little difficulty arises in judging with the microscope whether a mass is composed of one or more parts inferred to be crystal fragments. That a dispersion process will resolve this mass into these fragments is not apparent. When a small crystal is examined with a given microscope, the conclusion may not be drawn that there are no imperfections (i.e., cracks, etc.), for a more powerful one might show it to be faulty.

There is evidence from theoretical considerations based on molecular theory and thermodynamics to show that all lattice planes are not bound together

by the same force per unit area (7, 8). This structure accounts for the discrepancy between the calculated and observed breaking strength, the force in mechanical slipping, etc. It is also shown that crystal surfaces are covered by cracks criss-crossing them in an irregular mosaic pattern. Evidence has been obtained of these on etched crystals.

EFFECT OF COMPOSITION AND SIZE ON DISPERSION

Even if it were possible to prove the existence of a unique particle set for all known mineral species, it is still conceivable that the majority of products obtained on mixing these have no unique structure. In order that they have, it is necessary that the applied deflocculation process produce simultaneously complete dispersion in each of the component minerals. For particles of colloidal size, it would be surprising to find such a process. It is, perhaps, because of this fact that investigators in the United States proceed by attempting to disperse all particles having radiuses greater than an arbitrarily set value (6). This evades the difficulties of the colloidal range, but these processes are open to the same criticisms, though perhaps to a different degree, which may be raised against those designed to measure the whole prime structure.

CONCLUSIONS

It would seem that size distributions are comparable only when an inherent unambiguously defined particle set is placed in suspension in every case by the dispersion process. Such a set is assumed to be selected by the standard dispersion processes (3, 4) but the reality of a complete deflocculation seems to be assumed rather than proved. It is not the practice in scientific theory to introduce an unnecessary assumption even though it be designed in anticipation of future results. Theory and fact must grow together, and prime structure does not appear to be necessary to account for experimental facts.

There seems to be ample justification for establishing mechanical analysis upon the functional relationship between dispersion processes and size distribution. It seems that the term *duplicate* may be defined in a practical way so that the arbitrary selection of a dispersion process specifies a particle size distribution for every soil. A unique particle distribution of any kind should be discerned from a study of this relationship.

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THE EFFECT OF SOIL MOISTURE ON THE AVAILABILITY OF NITRATE, PHOSPHATE, AND POTASSIUM TO THE TOMATO PLANT[1]

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Considerable study has been made of the effect of soil moisture on nutrients in the soil, but little work has been done to determine the actual uptake of nitrate, phosphate, and potassium from soils of varying moisture content. This paper reports the effect of variations of soil moisture from 10 to 40 per cent on the amount of nitrate, phosphate, and potassium in the lower petioles of tomato plants grown on a uniform soil in which only the moisture was varied. The effect of soil water on moisture in the petioles, on soil nitrate, and on the plant growth, also is reported.

TREATMENTS

Two series of 10, 20, and 40 per cent moisture were made on each of two kinds of soil, making four independent tests of each moisture percentage. The first soil, called "station soil" in the tables, was taken from the horticultural farm. It is a brown to black clay loam. The second soil was made by mixing three-fourths station soil and one-fourth well-rotted compost.

METHOD OF DISTRIBUTING THE SOIL WATER

Hendrickson and Veihmeyer (5) have found it practically impossible to maintain a uniform soil moisture at a certain percentage. Added water caused saturation at certain points without becoming distributed throughout the soil. A special apparatus was set up which was found to distribute the water quite satisfactorily throughout the soil mass. Figure 1 illustrates the method used.

Funnels were connected with the glass tubes and one-third of the required water was added to each of the three soil layers, one-third being distributed over the top, one-sixth added to each of the short tubes, and one-sixth to each of the long tubes. The sand layer permitted the water to spread over the top of the soil as the water was poured in. It then distributed itself quite evenly through the soil. There was no concentration of roots at the sand layers, but the roots were distributed throughout the soil. This proves that the

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

moisture was distributed throughout the soil mass. Some difficulty was experienced from the clogging of some of the tubes, but a stiff wire plunged through the tubes cleared them so that the water entered readily.

METHOD OF MAINTAINING THE SOIL MOISTURE

The weight of air-dry soil contained in each pot was determined and the percentage of moisture in the air-dry soil was found by oven drying a sample at 100°C. The percentage of water to be added was based on the weight of oven-dry soil. For example, pot 3 contained 6,870 gm. of oven-dry soil. Since

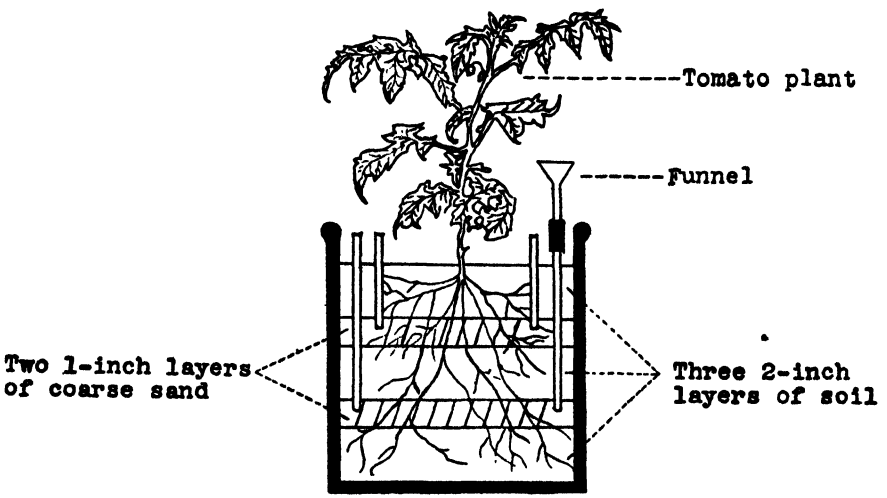

FIG. 1. CROSS SECTION OF 2-GALLON STONE JAR SHOWING APPARATUS FOR DISTRIBUTING WATER THROUGHOUT SOIL

it was to be maintained at 10 per cent moisture, 687.0 gm. of water should be present. The total weight of the complete pot was as follows:

	gm.
Weight of pot	3,880
Weight of sand	2,310
Weight of plant at setting	16
Weight of tubes	23
Weight of oven-dry soil	6,870
Weight of water which should be present	687
Weight at which the pot should be maintained to maintain 10 per cent moisture	13,786

As the plant grew, another plant of similar size was weighed and the weight added. The weight of a wire support was also added when the plant became too tall to remain upright alone.

In order to make sure that 10 per cent was not under the wilting point the wilting point of the soil was determined from the hydroscopic coefficient, as directed by Mosier and Gustafson (6). The wilting coefficient of the soil was found to be 8.2 per cent. When one-fourth compost and three-fourths soil were used the wilting coefficient was found to be 9.5 per cent.

Exactly 10 per cent moisture was not maintained continuously, since the pots were made to the determined weight only every 2 days. During that time about 50 gm. was lost at first. As the plant grew larger, sometimes 150 gm. would be lost, but rarely more than this. When 150 gm. was lost, only 537 gm. of water remained, or a percentage of about 7.8. This means that the moisture went from 10 per cent to about 7.8 per cent during the 2-day period, or that an average of about 9.0 per cent of moisture was actually maintained. The pots that had 20 per cent of moisture lost about 400 gm. every 2 days, when the plants were large. Since 1,374 gm. of water was present originally only 974 gm. remained at the end of 2 days. The actual precentage would then be 14.2 per cent, or an average of about 17 per cent. The 40 per cent moisture pots lost about 800 gm. and went to a minimum of about 28 per cent moisture, an average of about 34 per cent being maintained. Records of actual losses were kept, but were not important enough to be given here.

ANALYTICAL METHODS

Nitrate in the soil was determined by the phenoldisulfonic method, as described by Harper (4). Moisture in the petioles was determined by heating 10 gm. to constant weight at 80°C. Nitrate, phosphate, and potash in the plant were determined as previously described in other papers (1, 2, 3).

The samples for the determinations on the plant were taken from the large part of the petioles of the lower mature leaves, next to the main stem. Uniform samples were possible here, and the nearest approach to the actual nutrients flowing through the main stem could be had without destroying the plant. The last determination was made on the main stem, at the conclusion of the experiment.

PRESENTATION AND DISCUSSION OF RESULTS

Effect on growth characteristics of the plant

Table 1 shows the relative height, color, and green weight of the plants at the different amounts of moisture.

The 10 per cent soil moisture resulted in a dark green, woody, stunted plant. The dark green color evidently was due to a concentration of nitrate at the expense of further growth. The 20 per cent soil moisture resulted in medium succulence and good growth with a medium green appearance. The plants always looked healthy.

The 40 per cent soil moisture resulted in a very large, succulent vegetative growth. The plants were a lighter green color than with the 20 per cent mois-

ture. In several an unnatural yellowish color began to predominate toward the end of the experiment.

No conclusions could be drawn as to fruitfulness, since the volume of soil was not sufficient to carry the plants to normal maturity. Table 1 shows the green weight on the 40 per cent moisture pots to be about twice that on the 20 per cent pots, and four times that on the 10 per cent pots.

TABLE 1

Growth characteristics of plants

SERIES	SOIL MOISTURE*	SOIL	HEIGHT	COLOR OF FOLIAGE	GREEN WEIGHT
	per cent		*inches*		*gm.*
3	10	Station	34	Dark green	105
20	10	Station	36	Dark green	80
31	10	Station + compost	26	Dark green	65
45	10	Station + compost	29.5	Dark green	160
11	20	Station	46 5	Medium	240
21	20	Station	54	Fairly dark green	205
36	20	Station + compost	42	Medium	260
50	20	Station + compost	50	Medium	300
16	40	Station	62	Very pale green	200
25	40	Station	60	Medium	450
40	40	Station + compost	58	Light green	585
52	40	Station + compost	58.5	Light green	510

* The percentage of moisture is approximate.

Effect on moisture in the plant

The effect of soil moisture on that in the plant is shown in table 2. The plants in soil with 10 per cent moisture contained less water than those in soil with 20 per cent, except pot 11, and these, in turn, less than those in soil with 40 per cent. Evidently the plant endeavored, despite the low soil moisture, to maintain a normal moisture content, resulting in a stunted growth to counterbalance the small amount of moisture. The low moisture evidently hindered photosynthesis and consequently the supply of carbohydrates, resulting in little new tissue formation and an increase in nitrate concentration, due to a lack of its utilization.

Nitrate in the soil

Table 3 shows that the dry soil contained the largest amount of nitrate at the end of the experiment. The amounts of nitrate remaining in the 20 per cent and 40 per cent moisture pots were almost the same, the wettest soil having slightly more, with the station soil but slightly less when compost was added. This shows that the plant is able to take out nitrate to about the same

TABLE 2

Moisture content of plants

SERIES	SOIL MOISTURE*	SOIL	MOISTURE IN PLANT
	per cent		*per cent*
3	10	Station	90.8
20	10	Station	90.6
31	10	Station + compost	91.8
45	10	Station + compost	91 4
11	20	Station	90 7
21	20	Station	91 1
36	20	Station + compost	92 3
50	20	Station + compost	92.1
16	40	Station	93 5
25	40	Station	92 8
40	40	Station + compost	92 7
52	40	Station + compost	92 4

* The percentage of moisture is approximate.

TABLE 3

Nitrate nitrogen in soil

P.p.m. of dry soil

SERIES	SOIL MOISTURE*	SOIL	NITRATE N† FINAL
	per cent		*p.p.m.*
3	10	Station	25.6
20	10	Station	17 3
31	10	Station + compost	24 5
45	10	Station + compost	25.5
11	20	Station	13 4
21	20	Station	13 1
36	20	Station + compost	14 2
50	20	Station + compost	14 7
16	40	Station	14 2
25	40	Station	15 6
40	40	Station + compost	13 7
52	40	Station + compost	13 2

* The percentage of moisture is approximate.

† The initial nitrate in the station soil was 135 p.p.m., and in the composted soil 182 p.p.m.

level, irrespective of the amount of moisture present. It was not taken out of the dry soil because it was not utilized by the plant and became concentrated in the cell sap, which stopped its uptake by the plant.

Effect of soil moisture on nitrate in the plant

Table 4 presents the actual parts per million on the green weight basis of nitrate nitrogen found in the mature conducting tissues of the plant represented by the lower mature petioles of the leaves and by the entire lower stem at the end of the experiment. Logarithmic figures 2 and 3 represent the data of table 4. The values for January 4 used in the graphs are the average of the morning and afternoon determinations.

In the first stages of growth the plants on the 20 per cent moisture soils contained the most nitrate N in all groups but one, where that on the 40 per cent

TABLE 4

Nitrate analysis

P.p.m. of fresh tissue

POT NUMBER	SOIL MOISTURE*	SOIL	NITRATE IN PLANT					AVERAGE NO_3 IN PLANT†
			12/11/31	1/4/32 (a.m.)	1/4/32 (p.m.)	1/11/32	2/2/32	
	per cent		*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
3	10	Station	522	843	781	362	312	564
20	10	Station	558	694	1,190	500	...	729
31	10	Station + compost	906	481	658	500	500	609
45	10	Station + compost	1,035	781	833	500	500	730
11	20	Station	600	347	357	161	26	298
21	20	Station	690	280	609	161	...	435
36	20	Station + compost	1,021	624	641	274	62	524
50	20	Station + compost	1,208	694	694	454	34	617
16	40	Station	290	136	125	71	20	128
25	40	Station	763	448	675	125	...	503
40	40	Station + compost	906	367	581	147	25	405
52	40	Station + compost	906	347	347	109	24	347

* The percentage of moisture is approximate.

† The original nitrate in the plant was determined on a plant of the same size, age, and treatment as those used in the experiment. It was found to be 703 p.p.m. of fresh tissue. This figure was used in obtaining the average nitrate figures.

soil was highest (pot 25). Evidently nitrification was most rapid in the soil with 20 per cent moisture and the plant absorbed more. Soon, however, the stunting of plant growth on the 10 per cent moisture pots caused a lack of nitrate utilization, and nitrate accumulated, causing a dark green color and high nitrate level, as shown in the graphs. The plants in the 40 per cent moisture pot showed the lowest percentage of nitrate at nearly all times, showing either that nitrification was not so rapid in the wet as in the medium wet soil or that nitrate was utilized more rapidly by the plant in new tissue formation. The small increase in succulency and moisture in the plant could hardly account for

the decreased nitrate concentration. The graphs also show that nitrate concentrations become lower as the plant matures, although the stunted plants in the 10 per cent moisture pots maintained high nitrate levels to the end, as compared with the normal plants.

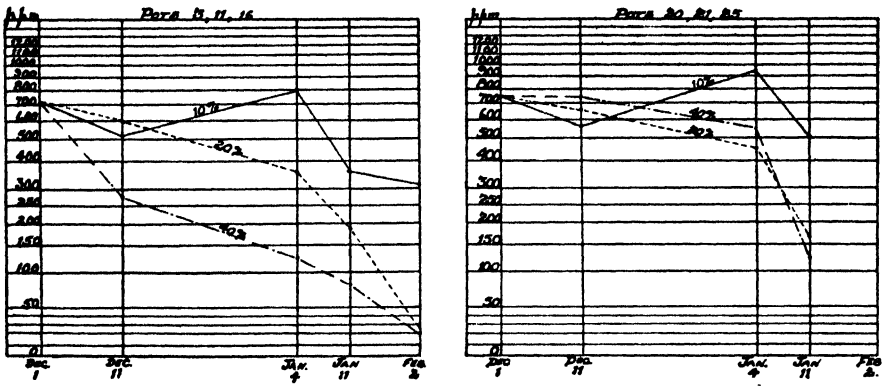

FIG. 2. NITRATE NITROGEN IN MATURE TOMATO PETIOLES GROWN IN SOILS CONTAINING 10, 20, AND 40 PER CENT MOISTURE
(Station soil)

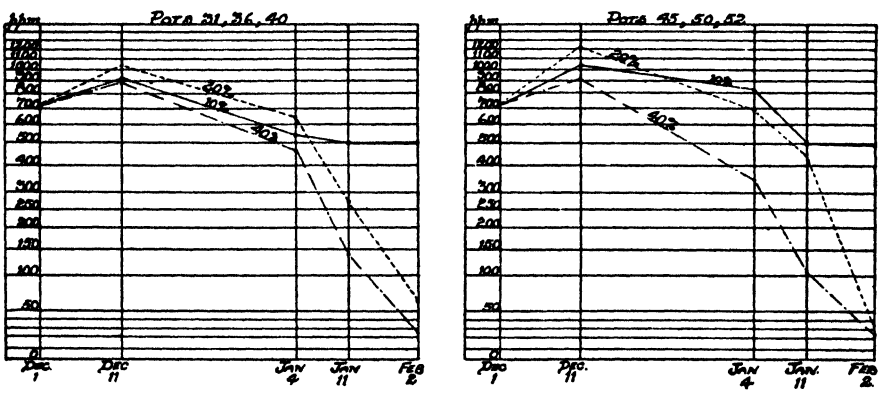

FIG. 3. NITRATE NITROGEN IN MATURE TOMATO PETIOLES GROWN IN SOILS CONTAINING 10, 20, AND 40 PER CENT MOISTURE
(Three-fourths station soil, one-fourth compost)

Effect of soil moisture on phosphate in the plant

Logarithmic figures 4 and 5 represent the data on phosphate found in the same sample taken for nitrate. The plants on the dry soil showed a little the most phosphate at first but as the readily available phosphorus was used the phosphate dropped until at the end of the experiment the curves in all four cases showed that a lack of moisture had a decidedly depressing effect on the

phosphate concentration in the plant. It will be noted that the 40 per cent moisture caused decidedly the highest concentration of phosphate, the 20 per cent moisture caused a medium amount, and 10 per cent decidedly the lowest amount in all four replications. The phosphate, contrary to the nitrate, increased toward maturity in all except the 10 per cent moisture pots. Evidently

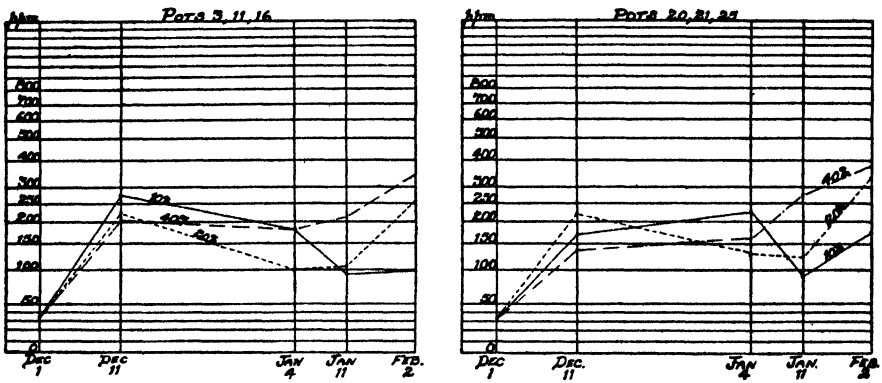

FIG. 4. PHOSPHATE IN MATURE TOMATO PETIOLES GROWN IN SOILS CONTAINING 10, 20, AND 40 PER CENT MOISTURE
(Station soil)

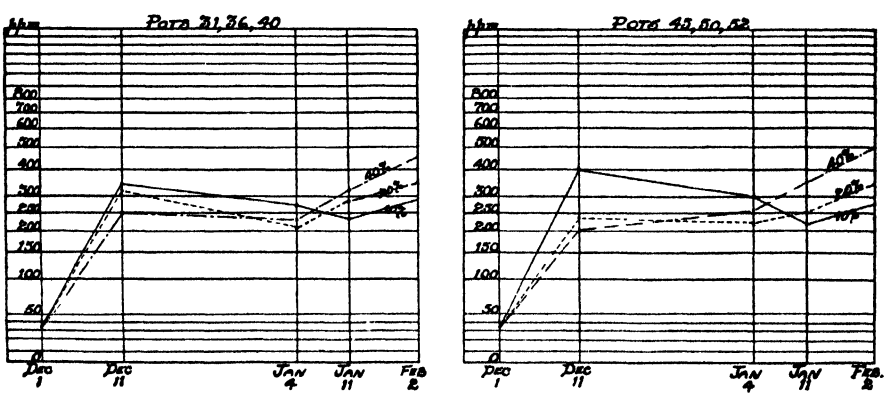

FIG. 5. PHOSPHATE IN MATURE TOMATO PETIOLES GROWN IN SOILS CONTAINING 10, 20, AND 40 PER CENT MOISTURE
(Three-fourths station soil, one-fourth compost)

the roots send up larger amounts of phosphorus when fruits are being set and matured. It seems probable that the lack of phosphate in the plant on the dry soil may be at least partially responsible for the accumulation of nitrate in these plants as previously noted. The lack of moisture also likely reduced carbohydrate formation and further reduced nitrate utilization.

TABLE 5

Phosphate analysis

P.p.m. of fresh tissue

POT NUMBER	SOIL MOISTURE*	SOIL	PHOSPHATE IN PLANT					AVERAGE PO_4 IN PLANT†
			12/11/31	1/4/32 (a m.)	1/4/32 (p.m.)	1/11/32	2/2/32	
	per cent		*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
3	10	Station	282	217	154	97	100	170
20	10	Station	168	277	166	97	173	176
31	10	Station + compost	331	294	235	232	298	278
45	10	Station + compost	403	333	266	229	266	299
11	20	Station	223	106	91	105	255	156
21	20	Station	226	166	87	126	323	186
36	20	Station + compost	328	208	200	286	344	273
50	20	Station + compost	238	250	174	250	333	249
16	40	Station	216	151	222	216	342	229
25	40	Station	146	200	125	258	370	220
40	40	Station + compost	251	263	190	320	482	301
52	40	Station + compost	206	294	210	345	500	311

* The percentage of moisture is approximate.

† The initial phosphate in the plant was secured on the same sample as used for nitrate. It was found to be 35 p.p.m. This figure was used in securing the average phosphate.

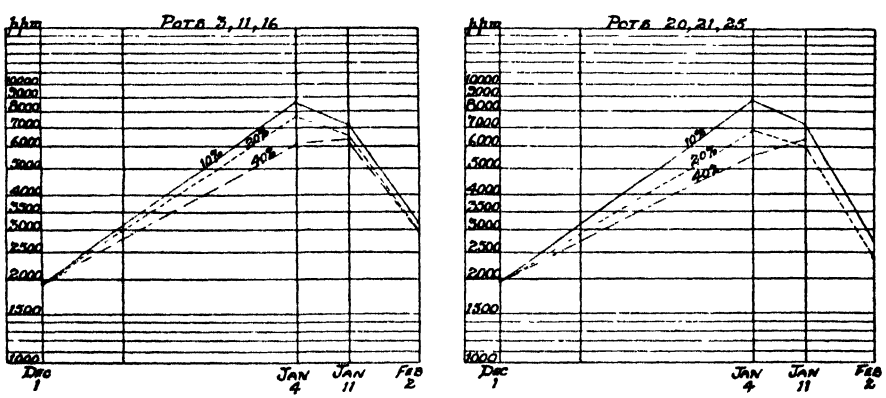

FIG. 6. POTASSIUM IN MATURE TOMATO PETIOLES GROWN IN SOILS CONTAINING 10, 20, AND 40 PER CENT MOISTURE

(Station soil)

Effect of soil moisture on potassium in the plant

Logarithmic figures 6 and 7 present graphically the results on potassium found in the same samples as used for nitrate and phosphate. Table 6 gives a tabulation of the actual parts per million of potassium in the fresh tissue.

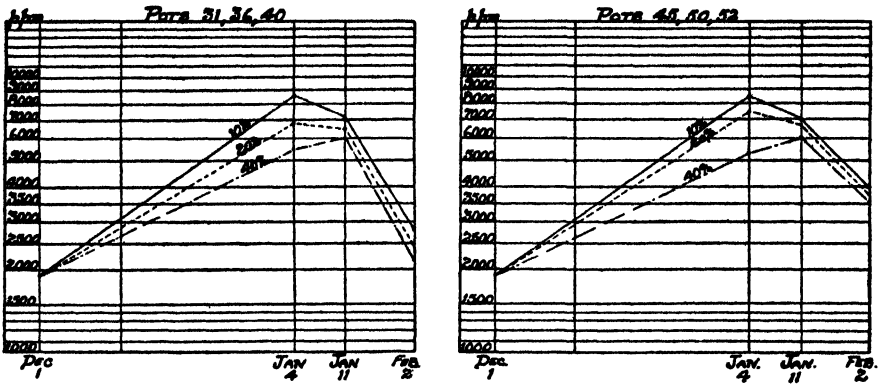

FIG. 7. POTASSIUM IN MATURE TOMATO PETIOLES GROWN IN SOILS CONTAINING 10, 20, AND 40 PER CENT MOISTURE

(Three-fourths station soil, one-fourth compost)

TABLE 6

Potassium analysis

P.p.m. of fresh tissue

POT NUMBER	SOIL MOISTURE*	SOIL	POTASSIUM IN PLANT				AVERAGE K IN PLANT†
			1/4/32 (a.m.)	1/4/32 (p.m.)	1/11/32	2/2/32	
	per cent		*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
3	10	Station	10,709	. .	7,111	3,250	5,396
20	10	Station	10,577	7,157	7,111	2,730	5,572
31	10	Station + compost	10,416	7,058	7,059	2,730	5,903
45	10	Station + compost	10,416	7,058	7,000	3,778	6,183
11	20	Station	8,928	6,666	6,857	3,000	5,144
21	20	Station	7,500	6,476	6,000	2,400	4,509
36	20	Station + compost	7,454	6,476	6,608	2,400	4,877
50	20	Station + compost	8,189	.....	6,800	3,636	4,817
16	40	Station	6,250	5,808	6,545	3,050	4,425
25	40	Station	5,357	5,866	6,476	. ...	4,434
40	40	Station + compost	5,147	6,181	6,117	2,235	3,965
52	40	Station + compost	5,147	. ...	6,000	3.652	3,755

* The percentage of moisture is approximate.

† The initial potassium in the plant was determined on the same sample as the nitrate. This was found to be 1,939 p.p.m. This figure was used in obtaining the average potassium.

The graphs are almost identical for the four replications. The concentration on the 10 per cent moisture pots was always highest, the 20 per cent moisture pots always medium, and the 40 per cent moisture pots always the lowest. The concentration of potassium rose to a peak and then fell off, probably as the fruits utilized it.

Evidently, lack of moisture did not retard potassium absorption. Since the concentrations did not vary greatly from one moisture percentage to another the fact that the concentration of soluble potassium undoubtedly was greater in the soil solution of the drier soils may have caused the increased concentration in the plants on these soils. The greater succulency of plants on the 40 per cent moisture pots may have caused a sufficient dilution of the cell sap to be partly responsible for the lessened potassium concentration in these plants.

SUMMARY

Soils were adjusted to approximately 10, 20, and 40 per cent moisture and tomatoes grown in them. The moisture was maintained and distributed through the soil by special apparatus and methods. Results are as follows:

Dry soil caused woodiness and a general stunting, with a dark green color.

Succulence increased, and deep green color decreased with increase of water in the soil.

Green weight was proportional to the amount of water.

Moisture in the plant varied with the moisture in the soil, although the differences were small.

More nitrate remained in the soils containing 10 per cent moisture than in the wetter soils. In all soils with 20 and 40 per cent moisture the nitrate was reduced to about the same level.

Twenty per cent soil moisture caused the highest nitrate concentration in the plant at the start and at nearly all times caused a greater concentration than did the 40 per cent moisture. The lower concentration in the 40 per cent moisture tests was likely due either to lessened nitrification in the soil with high moisture, or to more rapid utilization by the plant.

Ten per cent moisture soon caused an accumulation of nitrate in the plant resulting in an abnormally high nitrate concentration soon after the soil was adjusted to 10 per cent moisture. This abnormally high concentration was maintained until the end of the experiment.

Phosphate concentration in the plant varied directly with the percentage of soil moisture after the initial readily available supply was exhausted. Therefore, moisture supply directly influenced the availability of phosphate phosphorus.

The wetter soils maintained somewhat lower concentrations of potassium. This was likely due to the diluting effect and succulency of the tissue. The availability of potassium apparently was not favored by the presence of a large amount of soil moisture.

It seems that the major effects of low soil moisture are: (*a*) lessened nitrification in the soil but not lessened ability to absorb nitrate by the plant; (*b*) lessened ability of the plant to absorb phosphate and consequent nitrate accumulation in the tissue because of retarded tissue formation; and (*c*) a decreased water content in the plant which probably limits both photosynthesis and general metabolic processes in the plant.

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THE FOREST FLOOR UNDER STANDS OF ASPEN AND PAPER BIRCH

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The most widespread type of forest in the Great Lakes region is the aspen, often somewhat mixed with the paper birch.[1] In connection with a project of the Lake States Forest Experiment Station on the possibilities of converting aspen-birch stands to more valuable tree species, samples of the forest floor were collected in 1925 from nine plots, on each of which detailed measurements of the forest stand were made and the volatile matter, nitrogen, lime, acidity, and moisture equivalent determined.

The first three samples are from adjacent permanent sample plots in the Chippewa National Forest near Cass Lake, Minnesota, where conditions were assumed to be uniform, and sample 4 is from a plot 6 miles to the south. The other plots are more widely separated (table 1), those at Bagley lying 40 miles to the west of Cass Lake, those at Ely and Gunflint 130 and 190 miles, respectively, to the northeast, and the one at Trout Lake, Wisconsin, 260 miles southeast. The latter three are within the area of Early Wisconsin and the others within that of the Late Wisconsin drift.

METHODS OF SAMPLING AND ANALYSIS

Each sample of the forest floor was from one square foot. This was measured out, cut around with a knife, and the material completely removed down to the mineral soil. In order to compute the amount of dry matter per unit area the whole sample was air dried and kept so until dried at the laboratory at 100°C. and weighed. It proved difficult to make an entirely satisfactory separation but extreme care was taken in all cases. All samples except sample 2, were taken of the surface 6 inches of mineral soil thus exposed. Nitrogen was determined by the Kjeldahl method, volatile matter by prolonged ignition at dull redness, and lime by extracting the ash resulting from this ignition with *aqua regia* and precipitating as calcium oxalate. The acidity was determined by the Truog method and the hydrogen-ion concentration by the gas chain electrometric method with a bubbling hydrogen electrode and constant mechanical shaker. The moisture equivalent was determined by the usual method, at 1,000 times gravity. On the samples of mineral soil the determina-

[1] Kittredge, J., and Gevorkiantz, S. R. 1929 Forest possibilities of aspen lands in the Lake States. Univ. of Minn., Agr. Exp. Sta. Tech. Bul. 60.

TABLE 1

Sources of samples and character of soil

SAMPLE NUMBER	LOCALITY	SECTION	TOWNSHIP NORTH	RANGE	SOIL
1	Cass Lake, Minn.	6	144	30 West	Fine sand
2	Cass Lake, Minn.	6	144	30 West	Fine sand
3	Cass Lake, Minn.	6	144	30 West	Fine sand
4	Cass Lake, Minn.	6	143	30 West	Fine sand
5	Ely, Minn.	8	63	12 West	Loamy sand
6	Gunflint, Minn.	9	64	1 West	Loam
7	Bagley, Minn.	24	147	38 West	Fine sand
8	Bagley, Minn.	22	147	38 West	Fine sand
9	Trout Lake, Wis.	32	52	6 East	Loamy sand

TABLE 2

Properties and composition of the forest floor

Sample numbers are arranged in order of basal area

SAMPLE 2	SAMPLE 1	SAMPLE 6	SAMPLE 7	SAMPLE 8	SAMPLE 3	SAMPLE 5	SAMPLE 9	SAMPLE 4	MAXIMUM	MINIMUM
Age of trees in years										
38	38	40	30	40	38	41	19	30	41	19
Basal area, per acre in square feet										
111.9	110 3	106 3	105 8	103 8	93 8	91 5	86 1	51 9	111.9	51.9
Crown density										
0 6	0.6	0.7	0.8	0.9	0.7	0 5	0.8	0.3	0 9	0.3
Weight per acre of oven-dry forest floor, in pounds										
43,000	37,960	25,050	29,600	36,200	42,300	27,000	39,800	8,340	43,000	8,340
Organic matter (volatile matter) per cent										
45.2	64.7	56.2	65.7	69.1	52.9	56.5	44.3	46.9	69.1	44.3
Weight per acre of organic matter, in pounds										
19,436	24,560	14,078	19,447	25,014	22,377	15,255	17,631	3,911	25,014	3,911
Nitrogen in dry material, per cent										
0.87	1.49	1.22	1.63	1.63	1.14	1.16	0.91	1.20	1.63	0.87
Nitrogen in organic matter										
1.93	2.30	2.17	2.48	2.36	2.15	2.05	2.05	2.56	2.56	1.93

TABLE 2—*Concluded*

SAMPLE 2	SAMPLE 1	SAMPLE 6	SAMPLE 7	SAMPLE 8	SAMPLE 3	SAMPLE 5	SAMPLE 9	SAMPLE 4	MAXIMUM	MINIMUM
Nitrogen per acre, in pounds										
374	565	306	482	590	482	313	362	100	590	100
Nitrogen in surface 6 inches of soil, per cent										
. . ....	0.08	0.11	0.06	0 09	0.19	0.07	0.07	0.05	0.05	0 19
Lime in oven-dry material, per cent										
1.73	2.94	2.42	4 02	4 01	1.79	1.75	1 45	2 25	4 02	1 45
Lime computed to organic matter, per cent										
3.83	4 54	4 31	6.12	5 80	3.38	3 10	3.27	4 80	6 12	3.10
Lime per acre, in pounds										
744	1,116	606	1,190	1,452	757	472	578	188	1,452	188
Moisture equivalent										
84	144	105	116	127	82	105	112	84	144	82
Acidity, by Truog method										
Medium	Slight	Very slight	Very slight	Very slight	Medium	Slight	Medium	Very slight	Medium	Very slight
Acidity of underlying 6 inches of soil, by Truog method										
. . .	Medium	. .	Slight	Slight	. . . .	Strong	Very strong	Medium	Very strong	Slight
pH value										
5 6	5 8	5.7	6 3	6 4	5 4	5.6	5 2	5 9	6 4	5 2
pH value of underlying 6 inches of soil										
. . .	4.4	.	5 4	5.4		4 7	4 4	4 9	5 4	4 4
H-ion concentration ($C_H \times 10^{-7}$)										
25	16	20	5	4	40	25	63	12	63	4
H-ion concentration of underlying soil										
. . . .	400	. . . .	40	40	. .	200	400	125	400	40

tions were limited to nitrogen, acidity, hydrogen-ion concentration, and moisture equivalent. The results are given in table 2.

AMOUNT AND CHARACTER OF FOREST FLOOR

Amount

The amount per acre varied from 8,340 pounds of oven-dry material in sample 4 to a maximum of 43,000 pounds in sample 2, both from near Cass Lake but taken 6 miles apart. In samples 1 and 2, taken adjacent to the latter, the amounts were very similar, viz. 37,960 and 42,300 pounds.

Volatile matter

The volatile matter in the oven-dried samples varied from 44.3 per cent in sample 9, from Trout Lake, to 69.1 per cent in sample 8, from Bagley. Almost as wide extremes are shown by two adjacent plots at Cass Lake, samples 1 and 2, in which the percentages of volatile matter were 64.7 and 45.2, respectively. The weight of volatile matter per acre varied from 3,911 pounds in sample 4 to 25,014 pounds in sample 8.

Nitrogen

The nitrogen in the oven-dry samples varied from 0.87 per cent in sample 2 to 1.63 in samples 7 and 8. Here also the samples from adjacent plots, samples 1 and 2, show almost the extremes, viz. 1.49 and 0.87 per cent.

Nitrogen in volatile matter

When the nitrogen is computed on the basis of the volatile matter the range is less, being only from 1.93 to 2.56 per cent, with 2.30 per cent in sample 1 and 1.93 in sample 2. The amount of nitrogen in the forest floor per acre varied from 100 pounds in sample 4 to 590 pounds in sample 8.

Lime

The lime content of the dry material varied from 1.45 per cent on sample 9, from Trout Lake, to 4.01 and 4.02 in samples 7 and 8, from Bagley, whereas on the basis of the volatile matter the range was from 3.10 to 6.12 per cent. All are far above the minimum values found for peats that are well enough supplied with lime for the most calciphile cultivated crops.[2]

Moisture equivalent

The moisture equivalents, unlike the chemical values and acidity, were determined on the unground but well-mixed samples. They varied from 82 to 144, the extremes being found on the group of adjacent plots at Cass Lake.

Acidity and hydrogen-ion concentration

The determinations by the Truog method showed only very slight to medium acidity, whereas the electrometric determinations gave pH values of 5.2 to 6.4,

[2] Alway, F. J. and Nygard, I. J. 1927 Differentiation between lime deficiency and acidity in the case of peat soils. *Proc. First Internatl. Cong. Soil Sci.*, 2: 22–44.

or $C_H \times 10^{-7}$ values of 4 to 63. Thus none of the samples showed any decidedly acid condition. The surface of the underlying mineral soil was in all cases decidedly more acid, with a hydrogen-ion concentration in general about 10 times as great as that of the overlying forest floor, the $C_H \times 10^{-7}$ values of the six samples examined showing a range from 40 to 400.

DISCUSSION

The comparatively slight variations in the properties of the nine samples of forest floor make positive conclusions difficult other than the uniformly high content of lime and moderate content of nitrogen in the volatile matter (table 3). Sample 4, from the stand which had the lowest basal area and the lowest crown density, had much the lowest weight per acre of forest floor—only one-

TABLE 3

Properties of the forest floor samples arranged in order of lime content of the organic matter, assuming that all of the lime is combined with this

SAMPLE NUMBER	CaO WITH VOLATILE MATTER	REACTION	VOLATILE MATTER	N IN VOLATILE MATTER	NITROGEN IN SAMPLE	LIME IN SAMPLE	MOISTURE EQUIVALENT
	per cent	*pH*	*per cent*	*per cent*	*per cent*	*per cent*	
7	6.12	6.3	66	2 48	1.63	4 02	116
8	5.80	6.4	69	2.36	1 63	4 01	127
4	4.80	5.9	47	2.56	1.20	2.25	84
1	4 54	5.8	65	2 30	1 49	2 94	144
6	4 31	5.7	56	2.17	1 22	2 42	105
2	3.83	5.6	45	1.93	0.87	1.73	84
3	3.38	5.4	53	2 15	1 14	1 79	82
9	3 27	5.2	44	2 05	0 91	1.45	112
5	3.10	5.6	56	2 05	1.16	1.75	105

third as much as the next highest, sample 6. The former plot had been burned over after the aspen started, 30 years before, which may account for the small accumulation of forest floor. On plot 65, at Bagley, also with 30-year-old trees, the amount of forest floor was three times as great. Low basal area and low age, at least down to 20 years, do not appear to result necessarily in low amounts of floor per unit area, as evidenced by a comparison of sample area 8 at Bagley with number 9 at Trout Lake. On the latter the amount of floor is a little the higher although the trees are scarcely half as old and have only four-fifths as great a basal area.

The properties, other than weight per acre, show no constant relation to age, basal area, or crown density of the forest cover. The lack of relationship is not only evident in general but also specifically on the three adjacent and similar Cass Lake plots, on which the floor varied in properties almost as much as between the most widely separated areas, as for example in the moisture equivalents and the percentages of volatile matter and nitrogen. The causes of the differences apparently are not to be found in the age or density of the forest

cover. Samples 5 and 9 from the area of the Early Wisconsin drift have lower percentages of lime than the others, all of which are from the very calcareous Keewatin drift, a fact which suggests a relation to the mineral soil underlying the forest floor.

SUMMARY

Under stands of aspen-paper birch, the most widespread type of forest in the Great Lakes region, the forest floor was sampled in nine places. The amount per acre varied from 4 to 21 tons, the content of volatile matter from 44 to 69 per cent, and that of nitrogen from 0.87 to 1.63 per cent, corresponding to 2 to 12 tons of volatile matter and 100 to 590 pounds of nitrogen per acre. The percentage of nitrogen in the volatile matter varied from 1.93 to 2.56. The pH values ranged from 5.2 to 6.4 and the moisture equivalent from 82 to 144.

SOIL PROFILE STUDIES: V. MATURE PODZOLS

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One of the distinctive morphological features of a mature podzol is the bleached horizon A_2. The origin and mode of formation of this horizon have been discussed in an earlier publication (2). Within the podzol zone not all of the soils exhibit a morphologically distinguishable A_2 horizon. Some of them show chemical evidence of podzolization only, as shown elsewhere (3). A more clear-cut picture of a podzol, its chemical, physical, and even biological characters is, however, to be drawn from a podzol soil which has a definitely developed, morphologically distinguishable A_2 horizon. Such podzols are to be found on the Lakewood and sometimes the Collington and even on the Sassafras series of soils in New Jersey. A description and analysis of two podzol profiles of the Lakewood series is the subject of this paper.

PROFILE 1

Geographic position of area. The area where the samples from profile 1 were taken is located between the parallels 40° 16′ and 40° 14′ north latitude and between the meridians 74° 08′ and 74° 06′ longitude, south of the Hockhockson Swamp. The profile cut was made near the road which leads southeast to Green Grove. The region where this area is located is known as the Freehold area in the soil survey of New Jersey.

Topography, geological, and climatic features. The topography is level with slight undulations, and the area under consideration is on the more elevated level which dips gently to the east and to the streams which feed the Hockhockson Swamp to the northwest.

Geologically the area is in the Coastal Plain Province, which consists of formations of unconsolidated almost horizontal beds of gravel, sand, sandy clay, and marls with a slight dip to the coast. These formations are chiefly Tertiary, the upper Miocene.

The climate of the region is characterized by the relatively narrow daily and monthly ranges of temperature. The rainfall is rather heavy, averaging about 50 inches annually, with a minimum of about 40 and a maximum of over 70 inches. The mean annual temperature is about 53°F. with an absolute minimum of −11°F. and an absolute maximum a little over 100°F. During

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

the winter the ground freezes a few inches deep, but it does not remain in that condition all through the winter. Frequent thawing out takes place during the winter. The humidity is relatively high, except during some periods during the fall. An important feature of the rainfall element of the climate is its relatively even distribution. All of the climatic factors are therefore conducive to a thorough leaching of the soil, which in turn is effective as far as the process of podzolization is concerned.

Vegetation. The area is covered in places with pure stands of pitch pine (*Pinus rigida*), in all probability not the original timber. In places there is a mixed stand of pitch pine with black and white oak. An abundance of blueberry bushes (*Vaccinium*) is very characteristic of the area.

Description of soil profile

Ao: practically none, except for superficial debris of undecomposed leaves and twigs.

A_1: 13 cm. deep. Sandy material intermingled with some decomposed organic matter, light in color, structureless.

A_2: 25 cm. Bleached white sand, very distinct line of demarcation between the overlying A_1 and underlying B_1 horizons. In places it goes down into B_1 in pocket-like formation. No structure is apparent; it is slightly smeary when wet, dusty-like when dry. No visible traces of organic matter.

B_1: 20 cm. Light brown sandy material with tongues and pockets of white sand; it is slightly compacted showing indications of some structure, with a slightly heavier texture than A.

B_2: 35 cm. Light brown sandy material with darker (iron and humus) concretions scattered throughout. There are definite signs of a crumbly to a nutty structure with no indications of lamination. Like B_1 it is more compact than A_1 or A_2 with a slightly heavier texture than A.

C: A light brown fine sand, unconsolidated, with specks of mica scattered throughout, going down several feet deep.

It is clear that morphologically we are dealing with a genuine podzol of a mature age. And, of course, the chemical analyses show that the distribution of the chemical constituents in the profile is true to form for the podzolization process.

Ao layer. It is of interest to note that on the more elevated (microrelief features) spots of the area the Ao layer is feebly developed and in places there is practically none. This has been noted to be especially true in the pure stands of pine (usually pitch), but it has also been found to be true even in areas with a mixed stand of pine and oak (black and white). There is only a superficial cover of undecomposed and partly decomposed forest debris without the formation of a well-defined mat. Such observations have been made also with Dr. C. F. Marbut, whom the senior author had the opportunity to accompany during the summer of 1931, while he was studying the Freehold area and checking up some points with reference to the survey which was made earlier by Lee

and Tine (7). In his write-up, Marbut (7) refers to the peculiar condition of the Ao layer.

In connection with the process of formation of an Ao in the section under investigation, the frequent forest fires have to be considered. In the open areas the forest floor becomes thoroughly dry and is easily burned. This, of course, prevents the accumulation of organic matter. On careful examination one may find in the Ao-free area small depressions, in the more shady spots, a well-formed mat of organic matter. The damp conditions in these localized areas apparently protect the organic matter layer from the ravages of fires.

Chemical characteristics of profile 1

In table 1 the total analyses of the soil material in the respective horizons of the profile are presented.

SiO_2. True to form the A_2 horizon is highest in SiO_2, with a sharp decrease in the B horizon and an increase again in the C horizon. The data indicate a

TABLE 1

Total chemical analyses of soil profile 1 (mineral constituents)

LABORATORY NUMBER	HORIZON	DEPTH OF HORIZON	SiO_2	Fe_2O_3	Al_2O_3	CaO	MgO	K_2O
		cm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
78	A_1	13	97 16	1 3	0 407	0 118	0 0723	0.225
79	A_2	25	98 12	1 1	0 315	0 121	0 0795	0 200
80	B_1	20	93 32	1 9	2 50	0 124	0 1122	0 340
81	B_2	35	93 40	1 9	2 65	0 143	0 1157	0.365
82	C		95 97	1 35	2 09	0 203	0 1447	0.415

disruption of the silicate complex in the horizon of eluviation with the consequent loss of bases, splitting off of the sesquioxides, and separation of SiO_2. Hence an increase of SiO_2 in the horizon of eluviation, especially in A_2. The lower increase of SiO_2 in A_1 is apparent only since A, has a high organic matter content, in comparison with A_2, which is added to the mineral material from outside sources—the atmosphere and hydrosphere. There is, however, another important point to consider, namely, the fact that the disruption of the silicate complexes in A_1 is somewhat hindered because of the enrichment with Ca which comes from the mineralization of the organic matter increasing thereby the pH. True, the Ca remains there only temporarily, but it is apparently effective in preserving some of the silicate complexes. More evidence to that effect will be brought out in the discussion of the R_2O_3 data.

Not all of the SiO_2 released in the horizon of eluviation remains there. Some of it moves downward, as evidenced by the morphological features: tongue shaped protrusions and spots of bleached material in the B horizon. Thus the increase of SiO_2 in A_2 is not absolute but relative. The losses of bases and R_2O_3 exceed the loss of the SiO_2, and hence there is an apparent increase of SiO_2.

As we come to analyze the decrease of SiO_2 content in the B horizon, we must recognize again that it is only apparent, i.e., there is no actual loss of this constituent from the B horizon. The illuviation process brings about an increase of bases, sesquioxides, and various colloidal materials. These naturally decrease (relatively) the percentage of the total SiO_2 in the B horizon.

The increase of the SiO_2 content in the C horizon, as compared with B, is also only apparent. Since the parent material of this soil is geologically a transported material, the weathering forces as such were subdued at the time the soil forming processes came into play. Parent material from a geologically residual rock material is very strongly subject to the action of the forces of weathering, simultaneously with the forces of the soil forming processes. It is therefore logical to conclude that in the case under consideration the parent material has not undergone any very marked transformations ever since the soil forming processes set in.

Iron and aluminum. Mention has been made elsewhere (5) that in the circulation of the elements through the profile the sesquioxides are among those which do not return in any appreciable quantities as the bases do. Plants take up very little Fe and still less, if any, Al. As a result, one would expect a low sesquioxide content in the horizon of eluviation and an accumulation of these sesquioxides in the horizon of illuviation. This should be more true for the Al than for the Fe for two reasons. First of all, practically none of the Al is returned to the Ao layer through the plants. Secondly, the Al moves faster than the Fe because of the higher isoelectric point of Al. This has been brought out by the studies of Mattson (11), Joffe and McLean (6), and Joffe (4). The data in table 1 substantiate the statement made in the foregoing: more of the Al than the Fe has disappeared from A_1 and A_2, and relatively more Fe has accumulated in B.

The data on the sesquioxide content of the A_1 and A_2 horizons respectively are interesting. There are more Fe and Al in the A_1 than in the A_2 horizon. If it were the Fe alone one would be prone to ascribe the phenomenon to the return of this element by the organic matter of the accumulative Ao layer. Such an explanation, however, could not be accepted for the Al, which is not returned. It must be sought in the make-up of the silicate complexes and the degree of decomposition of these in the respective horizons, mention of which has been made in the discussion on the distribution of SiO_2 in the profile. The higher sesquioxide content in the A_1 horizon may serve as further evidence of the lower degree of disruption of the silicate complexes in the A_1 as compared with the A_2 horizon. There is also the possibility that the humus complexes in the A_1 tie up some of the sesquioxides.

In the analysis of any podzol the accumulation of the sesquioxides in the B horizon is very significant for a clear understanding of the process of podzolization. The accumulation of the sesquioxides represents the release of these from the horizon of eluviation in the process of humification and mineralization of organic matter and their precipitation in the horizon of illuviation because

of the higher concentration of bases and other electrolytes, which create the proper conditions—pH and other electrokinetic behavior—for the isoelectric state of these constituents.

An important function of the sesquioxides is their cementing action. Together with the fine particles which move downward mechanically they clog up the pore space making the B an impervious horizon. It is the cementing action of the sesquioxides which is responsible for the formation of orterde and ortstein.[2] A contributing factor in the retention of the sesquioxides is the irreversibility of the gels as they age or undergo the effects of drying. In a way the sesquioxides in the B horizon may be looked upon as the agents which prevent the further disintegration and decomposition of the soil materials, which makes this horizon a temporary storehouse of the substances used by the plant roots.

Calcium, magnesium, and potassium. A glance at the figures in table 1 on the distribution of Ca, Mg, and K in the profile shows that these three elements behave somewhat alike. About 50 per cent of each one of them has disappeared from the horizon of eluviation, when we take the percentage composition of the material from the C horizon as a basis for comparison. These three element show a relative increase in B, when compared with A, but not with C.

If we stop to analyze the data on these three elements separately, especially with reference to the B horizon, we may note that each one of them shows a behavior of its own. Thus the Ca shows the lowest accumulation (about 12 per cent when compared with A); Mg, a higher accumulation (about 44 per cent); and K, a still higher accumulation (about 66 per cent).

Two factors contribute to the low accumulation of Ca in B. First of all it enters into circulation through the profile to a greater extent than Mg because of the higher assimilation of Ca by plants. Secondly, the Ca is more readily leached and lost than the Mg, which might form new complexes of an insoluble nature and is apparently held more firmly in the complex.

The rather high K content in B and C is probably due to the presence of some greensand marl. This soil should therefore be classified as Collington following the nomenclature of the Soil Survey. There is not, however, enough of the glauconitic material in the parent material which could readily be recognized, and for that reason probably it has been mapped as Lakewood.

Distribution of organic matter and nitrogen in the profile

C:N ratio. In the soil microbiology literature on the organic matter content of the soil, the opinion prevails that the C:N ratio approaches, within limits, the figure 10. A great many deductions on the microbiological processes in cultivated soils have been made because of this ratio with no consideration to the intimate relationships existing between the horizons in the soil profile.

The data in table 2 reveal a decided variability of the C:N ratio in the profile.

[2] A more detailed discussion of ortstein formation may be found in a former publication (2).

The high ratio in A_1 is probably due to some charcoal which could be found all over the area as a result of the frequent forest fires. One should in general expect a high C:N ratio in this horizon because it is underlying the Ao layer and therefore receives much fresh organic matter, and besides it contains a lot of the fine roots of the herbaceous vegetation. Even though this profile showed but a feebly developed Ao layer, still there was enough to influence the percentage of the total organic matter and, of course, also the C:N ratio.

The C:N ratio in the other horizons is more significant. In A_2 it is lower, because of the high N content in the organic matter. Why there should be a high N content in A_2 will be discussed presently. The ratio goes up again in B_1, drops in B_2, and still more in C, where it approaches the generally accepted C:N ratio. It appears that in the sandy type of podzols the C:N ratio is far above 10.

TABLE 2

Distribution of organic matter and nitrogen in profile 1

LABORATORY NUMBER	HORIZON	DEPTH OF HORIZON	TOTAL C	TOTAL ORGANIC MATTER*	TOTAL N	N IN ORGANIC MATTER†	$\frac{C}{N}$ RATIO
		cm.	*per cent*	*per cent*	*per cent*	*per cent*	
78	A_1	13	0.75	1.303	0 0156	1 11	48.3
79	A_2	25	0.118	0.2034	0.0078	3.79	15.2
80	B_1	20	0 55	0.9482	0.0231	2 41	23.8
81	B_2	35	0.43	0 7413	0.0241	3.21	17.8
82	C	.	0 09	0.1542	0 0085	5.42	10.6

* Total C was multiplied by the factor 1.724.

† It is assumed that all of the N is in the organic matter.

Organic matter and N content. The outstanding feature about the organic matter distribution in the soil profile is the increase of it in the B horizon. Although there is no definite correlation between the R_2O_3 and the organic matter in the different horizons, it is apparent that an increase of one is followed by an increase of the other. This is controlled by the presence of other electrolytes and the pH. Apparently the pH at B is the isoelectric pH for the precipitation of higher amounts of humates. According to Mattson (11) at lower pH values a smaller proportion of R_2O_3 constituents is required for high humus fixation. If the R_2O_3 and humus complexes or a part of them in B are of such nature, then an increase in pH should release some humates. When the soil material was treated with a neutral salt at pH 7.0 for the determination of unsaturation, a release of humus substances took place as discerned by the color.

The R_2O_3 and humus accumulation in the B horizon is conducive to ortstein and orterde formation. Theoretically a higher pH should tend to form an iron ortstein and a low pH, a humus ortstein. More studies on the concretions of Orterde and of ortstein in podzols of various parent materials and pH are wanted to corroborate this.

True to form, horizon C has a very low organic matter content, but its adjacent position with respect to B_2, which has a high organic matter content, is favorable to the reception of some of the highly dispersed organic materials from above. Undoubtedly some of these organic complexes become more soluble at increased pH environments, which occur sometimes during the fall season, as pointed out elsewhere (5). These finely dispersed organic materials are high in nitrogen and, perhaps, this will explain the extremely high N content of the organic matter in horizon C.

As pointed out in the discussion on the $\frac{C}{N}$ ratio, the A_1 is contaminated with charcoal. This, of course, makes the total organic matter content appear somewhat higher than it actually is. It also brings down the percentage of N in the organic matter.

The low organic matter content in A_2 is reasonable in the light of the reactions that take place in this horizon. It is the horizon where most of the destructive or decomposition reactions take place in the process of soil formation. Morphologically one could hardly recognize the organic matter in this horizon. Charring the apparently pure white bleached sand brings out the fact that there is some organic matter and the chemical analyses prove that. However, there is a different type of organic matter in each one of these horizons. This is especially reflected in A_2. The organic matter is colorless and it is probably, as suggested by Williams (12), of the crenic and apocrenic acid type, which, by the way, contain 3 to 4 per cent N. This might explain the high N content in the organic matter of this horizon. It is very likely that the organic matter of this horizon is highly contaminated with fungus mycelium, since the reaction environment in this horizon is unfavorable for bacterial activity and favorable for fungi. It is to be recalled that fungus mycelium contains from 4 to 6 per cent nitrogen.

The high organic matter content in B is accompanied by a high percentage of N, as shown in table 2. The B_2 is higher in N than B_1. As explained in connection with the discussion on organic matter in horizon C, it is the degree of dispersion that controls the N content. The more highly dispersed particles are of a higher N content, and, with an increase in depth of the profile through B, there is an increase in the fineness of the particles. Edington and Adams (1) present results which show an enrichment of N in the B horizon of the podzol which they studied.

The total N content of the podzol soil under consideration follows the trend of the R_2O_3 or of the organic matter: lowest in A_2, increase in B, and a drop in C. The N content of A_1 is comparatively low because of the absence of an Ao layer, which usually increases the total organic matter content, and even though the percentage of N is as a rule low in A_1, its total N is high. In this case the results are obscured by the presence of charcoal, of which mention has been made.

Base exchange and unsaturation of soil

The podzol nature of this profile is very strikingly illustrated by the data in table 3, on the base exchange capacity and unsaturation of the soil in the various horizons. The A_1 horizon shows a rather high exchange capacity as compared with that of A_2 and C. As pointed out elsewhere (3), this is probably due to the relatively high organic matter content in A_1, higher than in any other horizon. Besides that, there seems to be, as pointed out in the discussion on the SiO_2 content, some mineral complexes in A_1, which are not disrupted and which probably manifest some exchange capacity. That most of the exchange capacity must be ascribed to the organic complexes is clear from a comparison of this property in A_1 and C, the latter having the smallest amount of organic matter. Only one-fifth of the exchange capacity in A_1 comprises the exchange capacity of C. The base exchange properties of organic matter had been discussed by McGeorge (9, 10).

TABLE 3

Base exchange and unsaturation in profile 1

LABORATORY NUMBER	HORIZON	DEPTH OF HORIZON	BASE EXCHANGE CAPACITY	UNSATURATION. REPLACEABLE H	UNSATURATION	REACTION	
						H_2O extract	Neutral salt extract†
		cm.	*m.e.**	*m.e.*	*per cent*	*pH*	*pH*
78	A_1	13	3.00	2 0	66 6	4 8	4 4
79	A_2	25	0.77	0 55	71 4	4 8	4.8
80	B_1	20	2.74	2.60	94 8	5.1	5.0
81	B_2	35	2 74	2 55	93 0	5 2	5.0
82	C	..	0 64	0 50	78 1	5 2	5 2

* m.e. = Milligram equivalents per 100 gm. of soil.

† A 1.0 *N* solution of neutral $BaCl_2$ was used.

It is of interest to note the low (as compared with A_2 and B) percentage of unsaturation in A_1: only 66.6 per cent of the base exchange capacity. Apparently some of the Ca, Mg, and K make up the rest of the exchange capacity. And yet its pH is lower than that of the other horizons, except A_2, which is the same as that of A_1.

In A_2 where the most destructive activity of the podzolization process takes place, the exchange capacity drops and the unsaturation increases.

An increase in exchange capacity and unsaturation takes place in B. The origin of the high base exchange capacity is to be sought in the colloidal inorganic complexes which accumulate in this horizon, in the probably new formations—complexes formed by the interaction of the sesquioxides and silica,— and in the organic matter, which is relatively high in this horizon.

A rather peculiar condition to be noted in B is the high unsaturation. It is very probable that this is due to the rapid circulation of the bases. They

seem to be removed rapidly and the H ions take their place. And yet the pH is higher in B than in A, an indication of a true podzol.

PROFILE 2

Geographic position of area. The area is located between the parallels 39° 46′ and 39° 48′ north latitude and between the meridians 74° 22′ and 74° 21′ at the junction of the roads from Cedar Bridge to Manahawken and Tuckerton. It is in the Chatsworth Area of the Soil Survey of New Jersey.

Topography, geological, and climatic features. The topography is generally level and in places hummocky. This localized area is dipping south and west toward the Wading River Valley, which eventually turns eastward—the general direction of the dip. It is about 120 feet above sea level.

The climatic features of the region are very similar to those of profile 1, except for the rainfall, which at the meteorological station in Tuckerton—about 20 miles south of Cedar Grove—is shown to be considerably lower than at Green Grove—the point where profile 1 is located. The mean annual precipitation is 44 inches and 50 inches respectively. The conditions are thus very favorable for the processes of podzolization.

Geologically this area is the same as the one of profile 1 with this difference: the sandy material from which the soil developed is only about 93 cm. deep and is underlaid by a heavy clay. The soil proper up to the C horizon is only 32 cm. deep, whereas in profile 1 the sandy material extends to a depth of several meters and the soil proper up to C is 93 cm. deep.

The vegetation. The area from which the soil sample was taken supports a poorly growing forest of pitch pine (*Pinus rigida*) about 40 years old. There is a dense understory of scrubby black oaks and the herbaceous vegetation is practically one hundred per cent blueberries and huckleberries (*Vaccinium* sp.).

Description of soil profile

Ao: very thin, consisting at best of sparse litter of the last leaf fall of pine and oak. The accumulation of organic debris in partial and advanced stages of decomposition, so characteristic of northern podzols under coniferous forest, is hardly discernible here or is completely absent. There are abundant signs in this region of recurrent forest fires to which may be ascribed the conspicuous dearth of surface organic matter.

A_1: 5 cm. deep. A fine, sandy, structureless material of a blackish gray color, with a considerable amount of incorporated organic matter, chiefly charcoal. The charcoal from forest fires is very evident in coarse particles and is chiefly responsible for the dark color of this horizon.

A_2: 15 cm. deep. A bleached, white sand, loose and structureless, forming a fairly uniform horizontal band in the profile and offering great contrast to the A_1 above and the B_1 below it.

B_1: 5 cm. deep A light brown, sandy horizon of heavier texture than the A horizons and showing more structure than the latter. Sieving showed the pres-

ence of numerous hard concretions of irregular form and from 0.2–1.0 cm. diameter.

B_2: 7 cm. deep. Of a lighter brown color than B_1 and with more concretions than the latter, but of similar structure and texture.

C: 61 cm. deep. A light yellow fine sand of loose structure and lighter texture than the B horizon. It rests upon a heavy clay in this locality.

As a result of the geological formations, as has been indicated, this profile is morphologically identical with profile 1, except for its depth.

Plate 1 is a photographic view of the profile. The clear cut A_2 horizon is typical of the mature podzols in this region. It shows the more pronounced structural constitution of the B horizon and below that the structureless C horizon. The underlying geologic clay formation is clearly shown in the picture.

Chemical characteristics of profile 2

In presenting the data on the chemical characteristics of profile 2, an attempt has been made to emphasize the outstanding differences between profiles 1 and 2, even though both are mature podzols of a very closely related physical, chemical, and morphological natures, and in the nomenclature of the U. S. Soil Survey both are classified as Lakewood sand. It is the finer differences in the make-up of the soil profile that are frequently responsible for certain behavior of the soil, especially its response in terms of vegetation or crops—the ulterior motive of our soil studies.

Table 4 presents the data on the mineral constituents of the soil profile.

SiO_2. Just as in profile 1, the SiO_2 is highest in A_2 with a sharp decrease in B and an increase in C. It is not necessary to repeat the course of the reactions which take place in the processes responsible for the differential distribution of the SiO_2 in the profile. The reader is referred to the discussion of this phase about profile 1. The relatively low SiO_2 content in A_1 is due to the high organic matter content, of which more is to be said presently.

Iron and aluminum. True to form, the horizon of eluviation (A_1 and A_2) is impoverished of the R_2O_3 constituents with an enrichment of the same in the horizon of illuviation (B_1 and B_2).

A distinctive difference, as compared with profile 1, is the higher accumulation of Al_2O_3 in B and the retention, if such were the case, of higher amounts of it in A, *notwithstanding the fact that the total R_2O_3 content in the parent material of these two profiles is not very far apart.* There is more than three times as much Al_2O_3 in the A horizon of profile 2 as in profile 1. Several explanations might be offered to account for this phenomenon. The most plausible one is that the underlying clay formation, to which mention has been made, prevents the rapid carrying off of the sesquioxides. Apparently they lag behind at certain periods and at times are perhaps carried to the upper horizon. This is more true of the Al_2O_3, which has a higher isoelectric pH than the Fe_2O_3, or in other words, it is more mobile. It is probable that the distribution of the Al in this

soil is one of the contributing factors in the unproductiveness of this soil where pitch pine and oak do not attain any considerable size. Further investigations would be necessary before this point could be established.

Calcium and magnesium. The striking feature about the two alkaline earth metals is the very low content as compared with profile 1. Strange enough, it is A_1 where more Ca is found. Undoubtedly this is due to the accumulation of Ca in Ao. The plants apparently gather all the Ca they can find and give it up again to the soil as the litter decomposes. It is very probable that the bases are held temporarily by the residual mineral complexes and also by the organic matter. In general, however, this soil is very low in Ca and this might be another direct and indirect contributing factor in its unproductiveness.

The Mg content is rather high as compared with the Ca content. Probably the parent material of this soil contains some very resistant Mg mineral. This might be inferred from the fact that there is very little translocation of Mg in the profile.

TABLE 4

Total chemical analyses of soil profile 2 (mineral constituents)

LABORATORY NUMBER	HORIZON	DEPTH OF HORIZON	SiO_2	Fe_2O_3	Al_2O_3	CaO	MgO
		cm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
83	A_1	5 0	90 50	0.72	1 25	0.123	0.119
84	A_2	15 0	97 00	0 69	1 07	0 076	.....
85	B_1	5 0	90 10	1 23	4 02	0.089	0.116
86	B_2	7 0	89 00	1.66	4 65	0 054	0.101
87	C	.	94 10	1 01	2 88	0.056	0.105

Distribution of organic matter and nitrogen in profile 2

C:N ratio. In table 5 the fact that the C:N ratio is far greater than 10—the commonly accepted ratio for cultivated soils—is brought out again. It seems that in this type of podzol (the tendency is probably true for any other mature podzol) such a ratio is typical.

The extremely high ratio in A_1 and A_2 is unquestionably due to the presence of charcoal, which accumulates in this region because of the forest fires. The high ratio in horizon C is difficult to account for.

Organic matter and N content. If we discount the factor of the charcoal in profile 2, the tendency toward accumulation of organic matter in B is also noted in this profile. The same is true if we critically analyze the N content. There is the tendency for a high N content in B, corroborating the analytical results of profile 1.

In general this profile shows a higher total organic matter and N content than profile 1. These follow the increase in R_2O_3 which corroborates the point made in this connection in the discussion of profile 1.

The data on the loss on ignition bring out the fact that, as a rule, this is not a very good index of the organic matter content of the soil.

TABLE 5

Distribution of organic matter and N in soil profile 2

LABORATORY NUMBER	HORIZON	DEPTH OF HORIZON	TOTAL C	TOTAL ORGANIC MATTER*	TOTAL N	N IN ORGANIC MATTER	$\frac{C}{N}$ RATIO	LOSS ON IGNITION
		cm.	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*
83	A_1	5.0	4.22	7.28	0.103	1.41	40.9	7.76
84	A_2	15 0	0.79	1.36	0.009	0 66	87 7	0.71
85	B_1	5.0	1.33	2.29	0.041	1.79	32.4	2.78
86	B_2	7.0	1.21	2.09	0 044	2.10	27.5	3.33
87	C	....	0.27	0.47	0.008	1.70	33.8	0.85

* Total C was multiplied by the factor 1.724.

TABLE 6

Base exchange and unsaturation in profile 2

LABORATORY NUMBER	HORIZON	DEPTH OF HORIZON	BASE EXCHANGE CAPACITY	UNSATURATION: REPLACEABLE H	UNSATURATION	REACTION	
						H_2O extract	Neutral salt extract
		cm.	*m.e.*	*m.e.*	*per cent*	*pH*	*pH*
83	A_1	5.0	11.5	9.55	83.00	4.2	3.4
84	A_2	15.0	1.8	1.05	60.00	4.6	4.2
85	B_1	5.0	7.2	6.50	90.30	4.9	4.6
86	B_2	7.0	6.6	6.30	95.40	5.0	4.8
87	C	....	1.8	1.20	66.60	5.2	5.0

Base exchange and unsaturation of soil

Nothing outstanding is to be inferred from the data in table 6. The reactions of base exchange and unsaturation in this profile are very similar to those of profile 1, and it is superfluous to follow these up again. The higher base exchange capacity and the high total unsaturation in B are easily explained. It is the higher organic matter content which is responsible for this phenomenon. The low pH values obtained by the neutral salt extract method is instructive, inasmuch as it shows the possibility of the hydrolysis effects of the possible Al salts.

Colloid fraction from profile 2

The colloids from the soil material of the various horizons were extracted and subjected to partial analyses with the purpose of establishing some relationships which might illustrate some reactions in the process of podzolization in a typical mature podzol.

The method of preparing the colloid was practically the same as that of the Bureau of Soils. The samples were sieved through a 2-mm. sieve, which allowed to pass almost everything except large woody particles. A 5-kgm. quantity from each horizon was placed into a 3-gallon bottle, 8 liters of distilled water added, and the reaction adjusted to be alkaline to litmus by adding 0.1 *N* NaOH. It was shaken vigorously and then allowed to stand for 24 hours, after which half of the suspension was decanted, water and NaOH added again, shaken, left standing 24 hours, and decanted again. This operation was repeated six times. The decanted suspensions were centrifuged, the colloids precipitated with $CaCl_2$, filtered through Chamberlain "F" filters, washed with H_2O, and dried on a steam bath with a very low flame. The dried material was ground in a mortar for analysis.

Table 7 gives the analyses on the colloid fraction.

SiO_2:R_2O_3. The ratio is highest in the horizon of eluviation and lowest in the horizon of illuviation. This is to be expected. The low pH in A indicates

TABLE 7

Colloid fraction of profile 2

	SiO_2	Fe_2O_3	Al_2O_3	$\frac{SiO_2}{R_2O_3}$	N	C	IGNITION LOSS	ORGANIC MATTER*	$\frac{C}{N}$ RATIO	N IN ORGANIC MATTER	CATION EXCHANGE
	per cent	*per cent*	*per cent*	*molar*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*m e.*
A_1	74 00	2 23	14 02	8 15	0 720	18 9	37 0	32 60	27 0	2 2	85 4
A_2	79 40	2 06	14.09	8 75	0 259	4 1	44 6	7 10	15 8	3 5	33 6
B_1	45.40	11 69	35 81	1 79	0 456	7 1	23.9	12 24	15 6	3 7	65 4
B_2	43.20	10 84	21 86	2 55	0 362	7.1	23 4	12 24	19 6	3 0	39 0
C	45.60	10 15	21 67	2 76	0 164	1 36	14 6	2 34	8 3	7 0	21 8

* The factor 1.724 was used to calculate the percentage of organic matter

a destructive acid hydrolysis whereby the sesquioxides are lost and the SiO_2 accumulates, as shown in the columns on the SiO_2, Al_2O_3, and Fe_2O_3 content of the colloids. This is also substantiated by the data on the total analyses of the soil itself in the respective horizons as shown in tables 1 and 4. On the other hand, the accumulation of sesquioxides in the horizon of illuviation (B) tends to decrease the SiO_2:R_2O_3 ratio.

It is questionable, however, whether the increase in the ratio of SiO_2:R_2O_3 in the colloid should be ascribed entirely to the colloidal complex. Undoubtedly some of the SiO_2 is not chemically combined with the sesquioxides as in the complex. There must be a certain limit for the quantity of SiO_2 which makes up the highest SiO_2:R_2O_3 ratio beyond which the SiO_2 is free and inactive. In his studies on the relation of base exchange capacity to the SiO_2:R_2O_3 ratio, Csiky[3] points out that when this ratio passes the value of 7.0, the base exchange capacity begins to drop. This may be offered as further proof that there is

[3] Csiky, J. S. 1932 Base exchange studies. Personal communication from author.

free SiO_2 mixed up with the complex and hence such a high ratio. What apparently happens is that in the process of the decomposition of the clay nuclei SiO_2 is released, but it remains behind in the A horizon. Some of it is in an amorphous form and in the process of colloid extraction it appears with the colloid complex, thereby increasing the $SiO_2:R_2O_3$ ratio.

If we accept the $SiO_2:R_2O_3$ ratio in C as a basis of comparison and remember that only small amounts of amorphous SiO_2 penetrate beyond the B horizon, then the ratios in B_1 and B_2 become significant. The oppositely charged R_2O_3 and SiO_2 colloids released by the forces of the podzol process of soil formation meet at B to become discharged and mutually coagulated giving rise to new formations. The disrupted mineral complexes from A_1 and A_2 because of the same forces attain colloidal dimensions, move downward to be "filtered" out at B. All of that makes the colloid fraction of this horizon more representative, less liable to foreign colloidal intrusions, except perhaps for some free Al_2O_3 and Fe_2O_3, and therefore the $SiO_2:R_2O_3$ ratio is closer to that of the soil colloid fraction. As we compare the ratios of C and B we note that in B it is narrowing. The absence, or rather limited supply, of bases in the soil material and the low pH are conducive to such a medium ratio, becoming narrower as the sesquioxides appear in a medium of a higher base content, or a higher pH.

The low Fe_2O_3 content in A_1 is indicative of the thorough podzolization of the soil under consideration. It is of interest that less Al_2O_3 has disappeared from the colloidal complex in A, as compared with C, than Fe_2O_3. It is probable, as suggested in the discussion on the total analyses of these constituents, that there is some return movement of Al_2O_3 because of the underlying geologic deposits.

Organic matter and N content. The colloid fraction of the organic matter is more instructive than the total organic matter. This is especially true for the A_1 horizon where large amounts of charcoal obscure the true picture of the organic matter in the soil. The method used in dispersing the colloids does not in all probability affect the charcoal, and organic matter content of the colloid fraction is therefore more representative.

The data show that the percentage of N in the organic matter of the colloid fraction is high in A_2. It corroborates the postulate made in the discussion on the organic matter of profile 1 that its nature varies with the horizon in the profile. What makes the C horizon so high in N is difficult to explain, except that the method of dispersion brings into the extract the very fine particles of the organic matter which are as a rule higher in N.

Closely related to the organic matter content is, of course, the C:N ratio. It shows a rather narrow ratio as compared with the C:N ratio on the total soil analyses. The charcoal factor, of course, needs to be considered in this connection. Besides, the method of dispersion does not apparently bring in all of the organic matter. It leaves a residue high in carbon and low in N. Only the fine particles which are higher in N enter into play. The narrower C:N ratio seems to prove this.

There is no close relation between the organic matter and the R_2O_3 in the colloid fraction, as there is in the total soil analysis. Again, this is probably due to the incomplete dispersion of the organic matter.

The data on the loss on ignition do not compare favorably at all with the data on the total organic matter. This discrepancy must be sought in the errors involved in the procedure of "the loss on ignition" rather than in the fact that the factor 1.724 which is used for converting carbon into organic matter may be perhaps too low, especially for the colloids where a high amount of organic matter is found. A discussion on the reliability of this factor has been presented by Lunt (8).

The data on the total N in the colloid fraction show the eluviation condition of A_2, accumulation in B, and a drop again in C. These are characteristic features of the constitution of a mature podzol.

Base exchange capacity of colloid fraction. The data on the base or cation exchange in the colloid fraction show wide variations in the profile, and yet the tendency is for a decrease in A_2 (the high capacity of A_1 is due to the large amount of organic matter), an increase in B, and a decrease again in C. A similar tendency is noted also in the analyses of the total soil.

GENERAL DISCUSSION

The similarity of the two profiles discussed in the preceding pages consists in their maturity, in the clear-cut differentiation of horizons with all the characteristic morphological features of a mature podzol. The chemical analyses corroborate the morphological features. There is, however, one distinctive difference between the two profiles: profile 1 is 93 cm. and profile 2 only 32 cm. deep—figuring the soil body from the surface to the bottom of the B horizon. As pointed out, the shallowness of profile 2 is due to the underlying geologic deposits of clay. Here is a case where the parent material inhibited the growth of the soil body. All other factors being equal, with the exception of the clay deposition where profile 2 developed, the forces of the climate and vegetation produced a deep soil body in one case and a stunted soil body in the other case. From the agronomic point of view the shallow profile means a lesser area of "feeding grounds" for the plants.

In speaking of the maturity of the profiles, it is worth while to take notice of the similarity in composition of B_1 and B_2. It indicates an equilibrium condition with respect to the formation of the horizon of illuviation. The natural course of the growth of this horizon is from the bottom up, and the homogeneity, as far as the chemical composition is concerned, is evidence of the completion of the process.

The maturity of the soil under consideration—the Lakewood series—has been favored by the rapidity and perhaps by the type of decomposition of the forest litter. The fact that large areas of this soil have practically no Ao layer is sufficient proof of the course of decomposition of the organic matter. Another factor contributing to the mature state of this soil is the low Ca content of the parent material, as pointed out earlier in this paper.

The value of total chemical analyses of soil has been repeatedly questioned, especially from the standpoint of soil fertility practices. It must be admitted, however, that from the pedogenic point of view, the total analyses are a great aid in elucidating the reactions which take place in the processes of soil formation. This has been clearly brought out in the presentation of the data on the total analyses of the soils and it has been corroborated by the analyses of the colloid fraction which shed no more light on what is going on in the soil than the total analyses did, except for the important property of the $SiO_2:R_2O_3$ ratio in the colloid fraction, especially in the B horizon. It is probable that by improving the technique of colloid extraction the analyses of the colloid fraction might mean more. As they are, they supplement very effectively the data on the total analyses.

SUMMARY

Data are presented on the morphology and chemical composition of two podzol profiles from the soils of the Lakewood series.

A distinctive morphological feature of the profiles is the practical absence of an Ao layer.

A discussion is presented on the distribution of the mineral constituents SiO_2, R_2O_3, Ca, Mg, and K in the profile.

The importance of the movement of Al_2O_3 and Fe_2O_3 in the profile is pointed out. It is to be noted that some of the R_2O_3 constituents present in the A horizon indicate a slowing down of the decomposition of the mineral complexes, which is due to the circulation of bases.

The Ca, Mg, and K behave somewhat alike with respect to their distribution in the soil profile. Ca shows a lower accumulation than Mg or K.

The distribution of organic matter in the profile is obscured with respect to the A horizon because of the presence of charcoal due to forest fires. The C:N ratio is not 10, the commonly accepted figure. It is a good deal higher. It is low in A_2, increases in B, and drops in C.

There is a high N content in the organic matter of A_2. It is pointed out that the type of organic matter varies in composition in the different horizons. It is suggested that the crenic and apocrenic acids in A_2 and the prevalence of fungi in A_2 might account for the high N content in the organic matter. There is also an accumulation of organic matter in the B horizon.

The podzol nature of these profiles is very lucidly illustrated by the base exchange capacity and unsaturation in the various horizons.

The colloids from profile 2 have been extracted by the Bureau of Soils method. One of the most important features of the analyses of the colloid fraction is the $SiO_2:R_2O_3$ ratio, which approaches the figure 2 in the B horizon. In general, however, the analyses of the colloids supplement the data on the total analyses.

The soils investigated are mature podzols and both profiles are identical, except that profile 2 is shallower because of the geologic deposits of clay under-

lying the sandy parent material. This caused what was termed a "stunting effect" on the growth of the soil body.

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PLATE 1

THE CONSTITUTION OF PROFILE 2

PLATE 1

SIXTEENTH SESSION OF THE INTERNATIONAL GEOLOGICAL CONGRESS

The third circular for the sixteenth session of the International Geological Congress, which is to meet in Washington, U. S. A., from July 22 to 29, has been issued. It contains full information about meetings and about excursions, with costs. Before the Congress there are excursions to various parts of the eastern United States, lasting from 4 to 12 days, and a transcontinental excursion eastward from San Francisco for those coming to the Congress from the west. For those arriving at New York too late to take part in these longer excursions there will be a number of short trips to nearby areas of geologic interest.

Alternate days during the sessions of the Congress will be given to excursions to areas around Washington.

After the sessions there will be two longer transcontinental excursions, each lasting 31 days, and two shorter excursions, one for the study of the glacial geology of the Central States, the other for the study of the pre-Cambrian area, including the iron and copper deposits, of the Lake Superior region.

In order to make these excursions generally available, it has been possible, through the generous assistance of the Geological Society of America, to offer the longer excursions at a considerable reduction below actual cost.

For special discussion at the scientific sessions in Washington the following topics are announced:

Measurement of geologic time by any method.
Batholiths and related intrusives.
Zonal relations of metalliferous deposits.
Major divisions of the Paleozoic era.
Geomorphogenic processes in arid regions and their resulting forms and products.
Fossil man and contemporary faunas.
Orogenesis.
Geology of petroleum.
Copper resources of the world.

Membership in the Congress is open to any one interested.

For a copy of the third circular or other information address W. C. Mendenhall, General Secretary, U. S. Geological Survey, Washington, D. C.

INFLUENCE OF CROP RESIDUE DECAY ON SOIL NITRATES[1]

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Records show that for centuries man has recognized that crops following many legumes resulted in larger yields than when the preceding crops were non-legumes. Farmers in the timothy and red clover sections of the United States realize that the clover sod gives better results than timothy sod. Various reasons have been suggested. Some claim that more organic matter is added by the clover sod and appear to think that this accounts for the beneficial effect. A common belief is that the clover sod supplies large amounts of nitrogen. Others claim that the deeper root system of the clover brings mineral plant food constituents to the surface.

During the past few years agronomists have begun to attribute the greater immediate value of clover residue over that of timothy and other non-legumes in part at least to the effect on the availability of the nitrogen.

The present work was undertaken to throw some additional light on the perplexing problems of why crops following red clover should yield more than those following timothy or corn. In carrying out this study time did not permit an investigation of all of the factors which might be responsible for the different residual effects of these crops. In fact, attention was concentrated on whether the amounts of clover, timothy, and corn residues left in the field after harvest were sufficient to effect the disappearance or accumulation of nitrate nitrogen. This necessitated obtaining an approximate idea of the amounts of these crop residues (roots and stubble) left in the field at harvest. After this information was obtained it was planned to use these same percentages of residues to soil under controlled conditions in the laboratory in order to determine the effect on the nitrate content of the soil.

The literature dealing with root development of field crops is extensive. Weaver (38) and Miller (23) have made fairly complete bibliographies. Most of the work has been done in the short and tall grass soil sections of the United States. Here the lower soil horizons, according to Marbut (21), are not unlike the surface soils in texture and in structure. Consequently, King (14), Hays (6, 7), Ten Eycke (29, 30), and Weaver (38) have found extensive root development of many cultivated crops below depths of 2 feet.

The soils in the humid timbered soil section of eastern United States as a group are charac-

[1] Thesis submitted in partial fulfillment of the requirement for the degree of doctor of philosophy at Cornell University.

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terized by shallow surface and compact heavy lower horizons. McCool (20) states that there is a marked difference both in weight and in length of roots on different soil types. Carlson (2) noticed that in compact soils all strains and varieties of alfalfa developed branch roots. Laird (15) found that the percentage of Bermuda grass roots found in the upper 8 inches of soil increased very markedly as the clay content of a sandy soil was increased.

The work of Goff and Beckwith (5) and Jones (13) in New York, Hickman (9) in Pennsylvania, Newman (24) in Alabama, and Woods (43) in Connecticut tends to prove that the main development of roots of nearly all cultivated plants in the eastern part of the country are in the upper 3 to 10 inches of soil.

The disappearance of all or a portion of the nitrates from the soil following the incorporation of crop residues of high carbon and low nitrogen content has been shown by a number of investigations. The literature on this subject has been thoroughly reviewed by Collison and Conn (3), Lyon (16), and Waksman (33) to 1927. Rahn (25) states that the addition of large amounts of energy-rich material causes an increase in the number of soil microörganisms. The microörganisms utilize the soluble nitrogen and convert it into protein. Rahn's explanation seems to be the one accepted by the majority of those who have worked on this subject. Lyon and Bizzell (18), Lyon, Bizzell, and Wilson (19), Wilson and Wilson (41), Holtz and Singleton (10), and Martin (22) have tested the effect of crop roots on nitrification in one way or another and agree that such materials do effect the accumulation or disappearance of soil nitrates.

Collison and Conn (3) believe that in addition to the disappearance of nitrates there is a toxic chemical agent which acts immediately upon plants after germination following applications of straw and other plant residues. This toxic idea has been dispersed pretty well by the work of Viljoen and Fred (32) and Blair and Prince (1).

Studies made by Waksman and Tenny (35, 36, 37), Waksman and Skinner (34), Whiting and Richmond (40), Wilson and Wilson (42), Jensen (12), Heck (8), and Rege (26), tend to show that the carbon-nitrogen ratio alone of materials does not tell the whole story regarding the relative effect on nitrification when incorporated in the soil.

Two bulletins published in 1930 tend to emphasize the practical importance of the subject under consideration. Lyon (17) and Collison and Mensching (4) give figures that indicate that legume roots and stubble stimulate the yield, whereas timothy residues depress the yield of crops that follow.

EXPERIMENTAL

The present experiment was designed to determine whether the roots and stubble of red clover, maize, and timothy are present at harvest in sufficient amounts to cause a marked change in the nitrate content of a soil.

The percentage of the residues (roots and stubble) in the soil for each of the crops was first determined, then the effect of field amounts of residues on nitrification was determined in the laboratory under controlled conditions, in three series of experiments. In the first series the field amounts of clover were taken as the basis of the amount of materials to be added. Similar amounts of nitrogen were applied in the form of timothy and maize residues. The second series was based on the amount of timothy residues (roots and stubble) found in the field. Here the effects of the two residues were compared when equal carbon amounts of each were added. In the third series a comparison of the effect of the two residues was made, where the carbon-nitrogen ratios of the two were made equal by the addition of nitrate of soda to the tumblers of soil receiving timothy residues.

Another set-up consisted of determining the effect of applying the water-soluble constituents from the residues of each of the three crops.

In addition to the determination of nitrates, total bacteria counts were made and the carbon dioxide evolved was measured. A test for vanillin in the residues of the crops used was made. General plant analyses of different kinds were also included in the experiment.

A preliminary study[3] consisted of checking at definite periods the change in soil nitrates caused by the addition of varying amounts of corn roots to soil, starting with a quantity too small to have any material effect upon the nitrates of the soil, then using a number of increasingly larger amounts until a change was produced. Similar amounts of nitrogen were added to soil in other tumblers in the form of dried blood. This afforded a comparison between the effects of additions of materials with widely varying carbon-nitrogen ratios.

The surface soil of the Hagerstown silt loam taken from virgin land lying adjacent to the experimental orchard on the farm of the Pennsylvania State College was used in this experiment. Mechanical, chemical, and mineralogical analyses of soil from this same place have been reported by Thomas (31).

In obtaining this soil the top inch of sod was discarded. The soil immediately below was screened through a 2-mm. screen into large galvanized iron cans, which were kept tightly closed. Since this experiment covered a long period, soil was taken on several different occasions. When it was too moist to be screened in the field it was spread in a thin layer on oilcloth in a building until it was sufficiently dry. At no time was thoroughly air-dried soil used. Before any experiment was set up the moisture content of the soil was always determined.

The lime requirement of the soil according to the Truog method showed it to be nearly neutral. The pH of the soil was slightly above 7.5. The addition of 0.3 gm. of calcium carbonate to 100 gm. of soil failed to affect the reaction according to pH determinations.

Methods of determining weights of roots and stubble

The method used in collecting the root and stubble data on the timothy and red clover fields was a modification of several methods. Laird (15) recently reported using a somewhat similar method. Galvanized iron cylinders, 8 inches deep and 8 inches in diameter, reinforced with a heavy iron hoop at the top were driven about 7 inches into the soil at representative spots in the field, the tops of the plants having first been cut so as to leave about 2 inches of stubble.

Next the full cylinders were dug out of the soil, weighed, and placed in closely fitting wire baskets (8 meshes to the linear inch), which were then placed in a tub of water. In a short time the cylinders were easily pulled off, leaving the soil columns in the baskets.

[3] This preliminary study was used as a portion of a thesis for a master of science degree at the Pennsylvania State College; since it has a direct bearing on this problem and has never been published, it is presented as part of this paper.

The baskets of soil were set in 2-mm. soil screens, many short pieces of wire were pushed through them from one side to the other. The stubble in each basket was attached by means of a cord to some of the top wires. These precautions prevented the mass of roots and stubble from falling into the bottom of the basket. The soil was washed away with a gentle stream of water. After the crop residues were removed from the baskets, they were spread between blotters to dry over night. The next day after foreign organic matter had been removed they were placed in paper bags until thoroughly air dried. Moisture determinations were made on the soil samples collected for this purpose when the cylinders were dug from the soil. The average weight of the soil in the first 11 cylinders weighed was used as the weight of the soil cylinders thereafter.

When the weight of dry matter in roots and stubble in a cylinder of soil and the weight of the soil and the percentage of water in it were known, the percentage of dry matter in the soil was easily figured. The weight of the residue per acre was figured directly, using the area of the top of the cylinder. The subsoil root weights of clover were obtained in two places by the method just described. The cylinder driven into the subsoil was a small iron one 6 inches in diameter and 6 inches deep. The chances of error in obtaining weight of subsoil roots seemed greater than in the surface soil.

An approximate idea of the amount of maize roots and stubble was obtained by digging a trench about 3 feet deep, cutting a row of maize hills through the middle. The surface soil was washed with a hose from the portion of the roots that remained in the soil. The weights thus obtained were multiplied by 2 to give the weight per hill. The weight per acre was obtained by multiplying the weight per hill by the number of hills in an acre. The acre weight of soil used in obtaining per cent figures was 2,000,000 pounds.

Air-dried roots and stubble of each of the three crops were ground in a Wiley mill and stored in stoppered containers for use in connection with the problem.

Preparation of soil cultures

The containers used for the nitrification studies were ordinary tumblers with tin pill boxes serving as covers. In case bacteria counts were to be made in addition to nitrates determined, 110 gm. of soil was used, otherwise only 100 gm.

Calcium carbonate (0.3 gm. per 100 gm. of soil) and finely ground dry organic materials were mixed with the soil prior to wetting. Sufficient distilled water was added to bring the total moisture content to 20 per cent (dry basis). An additional 5 ml. of water was used for each gram of crop residues applied. Where nitrate of soda or the extracts from plant residues were used they were applied in the water. The tumblers were kept in drawers in the laboratory and water was added from time to time as needed. The temperature during incubation averaged about 20°C.

The moist soil was placed in standard 2.5-liter acid bottles for making the carbon dioxide determinations. Each rubber stopper was fitted with two glass

tubes, the outer ends of which were kept closed by means of rubber tubing and pinch cocks. Except for the type of container and the amount of soil used, the preparations for the carbon dioxide and the nitrification studies were similar.

Methods of chemical analyses

The total nitrogen was determined by the official Gunning method; pumice stone was used instead of zinc dust. Nitrates were determined by the method of Schreiner and Failyer (27). The experiments were so set up that at each determination period three tumblers of soil for each treatment were used. The soil was mixed in battery jars with 500 ml. of water per 100 gm. of soil. Calcium oxide was used to help clarify the solution before filtering. In series 2 and 3 the soil was placed in percolation tubes with cotton plugs and water was added until 500 ml. had come through. In this case a saturated solution of lime water was used before clear water was added. The latter method was simpler than the former and seemed equally satisfactory. The total carbon analysis was made by the chromic acid method as perfected by White ahd Holben (39).

In series 1 the carbon dioxide was collected in soda lime by means of an absorption train similar to the one described by White and Holben in the total carbon analysis. In series 2 and 3 the carbon dioxide was collected in an excess of approximately 0.1*N* barium hydroxide, the excess being determined by titrating with 0.1*N* hydrochloric acid, phenolphthalein being used as an indicator. The apparatus for this purpose was designed by Merkle.[4]

In testing water extracts from plant residues for vanillin the resorcin test used by Collison and Conn (3) was employed.

The general plant analyses were made as described by Waksman and Tenny (36), 4 gm. of plant material being used instead of 2. Half of the cold-water soluble material was used for determining the soluble nitrogen and the other was taken down to dryness for soluble organic matter determination. The percentage of lignin, ash, and protein-free analysis was made according to the Schwalbe (28) method, duplicate samples being used to determine the nitrogen content. The hydrogen-ion determinations were made with a potentiometer.

Amounts of crop residues in soils

The figures showing the weights of the roots and stubble of the surface soil were obtained on the farms of the College of Agriculture at Cornell University. The soil was the Dunkirk silty clay loam as described by Howe, Buckman, and Lewis (11). The data were obtained early in July, 1926. In the opinion of those in charge of the farm operations this was an average season for the growth of timothy and clover. The timothy field was part of the Caldwell Experimental Field and had been fairly heavily fertilized. This was the second year timothy crop.

Table 1 shows that under the conditions described the average amount of timothy residue in the surface soil is over twice as great as the residue of clover.

[4] F. G. Merkle, associate professor of soil technology, Pennsylvania State College.

The same is true if we compare the largest number of pounds of residue of both crops or the smallest of each. It is of interest to note in this connection that the timothy root figures reported by Jones (13) agree with these fairly well. The cylinders used in getting the red clover data extended down to the heavy compact subsoil. The taproot of clover tapered rapidly and where it entered

TABLE 1

Amount of red clover and timothy roots and stubble in the surface soil of the Dunkirk silty clay loam

SAMPLE NUMBER	RED CLOVER ROOTS AND STUBBLE WATER-FREE BASIS		TIMOTHY ROOTS AND STUBBLE WATER-FREE BASIS	
	Pounds per acre	Per cent in the soil	Pounds per acre	Per cent in the soil
1	9,544	0.50	11,810	0.63
2	8,233	0.43	9,893	0.53
3	7,710	0.43	10,867	0.58
4	5,527	0.31	11,148	0.59
5	5,046	0.26	8,508	0.45
6	5,635	0.28	13,053	0.69
7	4,891	0.27	19,493	1.04
8	5,284	0.28	16,338	0.87
9	7,156	0.38	14,881	0.77
10	4,665	0.25	11,511	0.61
11	3,903	0.21	12,915	0.69
12	.. .	....	11,406	0.61
Average....	6,144	0.33	12,652	0.69

TABLE 2

Amount of maize residue left in the surface soil of the Hagerstown silt loam. Moisture-free basis

HILL NUMBER	PER CENT RESIDUE IN SOIL	POUNDS PER ACRE
1	0.053	1,052
2	0.059	1,182
3	0.057	1,133
4	0.044	885
5	0.041	829
6	0.04	805
7	0.048	961
Mean	0.049	978

the subsoil layer it split into two fine twine-like branches. The portion by weight of the plant that was found in the 6–12-inch layer of soil is comparatively unimportant: the weight of red clover roots was 134.7 pounds per acre; of this, 0.008 per cent and 0.0077 per cent, respectively, were found in this layer in two samples. A number of very fine thread-like timothy roots entered the subsoil layer. Their total weight was estimated to be very small.

In the light of this information we could not attribute greater crop yields following red clover than following timothy to greater amounts of organic matter added by the clover.

The data (table 2) pertaining to maize residue (roots and stubble) were obtained on the Hagerstown silt loam soil on September 25, 1926. This soil is underlain by a heavy clay or clay loam subsoil, which, in the place where the maize roots were collected, came within 7 inches of the surface. It was noticed here that only a very few fine maize roots entered the heavy layer. This point was also mentioned by Hickman (9) who worked on this same soil in 1887.

The maize, which was one of the tall growing varieties of dent, was just ripe. A fair crop of maize was obtained in central Pennsylvania that season.

TABLE 3

Analysis of residues and dried blood

MATERIAL	PER CENT MOISTURE-FREE BASIS		CARBON-NITROGEN RATIO
	Nitrogen	Carbon	
Clover residue	1.6707	38.4166	22.9:1
Maize	0.607	35.0965	57.8:1
Timothy	0.5257	42.4921	80.8:1
Dried blood	10.787	45.35	4.2:1

TABLE 4

Amounts of materials added in series 1

MATERIAL	LOW	MEDIUM	HIGH
	gm.	*gm.*	*gm.*
Clover residue	0.2	0.33	0.5
Maize residue	0.5502	0.9079	1.374
Timothy residue	0.634	1.0461	1.585

Series 1

Nitrification of residues. The nitrification studies in series 1 were based on the amounts of clover residues found in the field. The three amounts of this material used approximated the low, average, and high amounts found. In making up the maize and timothy residue soil mixtures the amounts added supplied the same quantity of nitrogen as were supplied by the clover residues. Table 3 shows the analysis of these residues and dried blood as to the total carbon and nitrogen.

The exact amounts of the different materials used per 100 gm. of soil on a moisture-free basis is shown in table 4.

The amounts of maize residue and the high amount of timothy materials added are higher than the amounts found in the field. The low amount of the timothy residue used is about the same as the average amount found in the

field, and the medium amount added corresponds very closely to the largest amount found in the field.

Table 5 shows that field amounts of clover residue caused a very slight depression in soil nitrates during the first period of incubation. Even this depression was broken by periods of slight accumulation of nitrates. All amounts of timothy residues used had a very pronounced depressing effect on soil nitrates; not a single determination showed an increase of nitrates over the corresponding no treatment. It is evident that where the amounts of tim-

TABLE 5

Variation of nitrates (NO_3) from no treatment

Expressed as parts per million of dry soil

INCUBATION PERIOD	TREATMENT PER 100 GM. OF DRY SOIL					
	Equal amounts of nitrogen		Equal amounts of nitrogen		Equal amounts of nitrogen	
	2 gm. of clover residues	Timothy residues	0.33 gm. of clover residues	Timothy residues	0.5 gm. of clover residues	Timothy residues
days						
1	−1.3	−11.0	−.8	−9	−4	−9
2	+1.9	−7.6	+2.2	−7	+20	−4
4	−.8	−20.6	−2.6	−20	−9	−19
7	−.9	−60.6	−11	−68	−27	−62
10	+16.7	−39.5	+29.4	−60	+3	−48
14	−24.1	−76.2	−5.2	−80	−6	−60
21	+19.8	−134.6	+9	−156	−28	−139
28	−46.7	−137.9	−34	−142	−26	−142
35	−56.3	−186	−24	−196†	−13	−136
42	−53	−130.8	−23	−173†	−10	−173*
49	−44.0	−200	−24	−231	+45	−236
56	−52.7	−229	−36	−273	+11	−289
70	−205.6	−266	−204	−355	−68	−381*
84	−14.7	−131	+11	−150	+10	−230
105	−7.3	−100	+18	−151	+35	−245
135	+4.0	−123	+26	−130	+24	−213
210	+43	−33	+120	−34	+34	−128

* All nitrates disappeared.

othy roots and stubble approach the amounts used in this experiment a prolonged depression of nitrates will follow. The larger the amounts of timothy residues added the longer was the depression period severe. In general the depression was very marked from the second to the tenth week. In the case of the two larger additions of timothy residues there was an entire disappearance of nitrates at times. The rather irregular fluctuation of the nitrate curves may be due in part to temperature variations in the laboratory.

The additions of maize roots and stubble corresponding to the three clover residue applications affected soil nitrates as shown in table 6.

There was a rather marked nitrate depression below the corresponding no treatment. As in the case of timothy the larger the amounts of material added the more lasting seems to be the severe depression period. In the case of the small application the depression was not continuous nor very marked except in the seventh-day determination.

None of the amounts of maize residues found in the field amounted to more than 0.06 per cent in the surface soil. The preliminary study (table 7) shows the effect of the addition of seven different amounts of maize roots on the soil nitrates. The smallest amount applied was 0.2 gm. to 100 gm. of dry soil. This amount appears not to have caused any nitrate depression during the first 12 weeks. The larger amounts of roots up to 1 gm. caused a slight nitrate depression which lasted from 6 to 12 weeks. When amounts of more than 1 gm. were used the depression was more noticeable. In this work stubble was not

TABLE 6

Effect on soil nitrates of adding varying amounts of maize residue

Results expressed as a variation of nitrates in parts per million of dry soil, from no treatment.

INCUBATION PERIOD	TREATMENT PER 100 GM. OF DRY SOIL		
	0.5502 gm.	0 9079 gm.	1 374 gm.
days			
1	14	30	30
2	6	−45	−70
4	65	85	55
7	−148	−135	−117
10	−64	−59	−266
14	−17	−43	−126
21	7	−75	−207
28	−14	−14	−81
42	28	−57	−147

mixed with the roots. The addition of a material with a very narrow carbon-nitrogen ratio like dried blood (table 8) tended to increase nitrate accumulation.

Since the percentage of roots and stubble in the field is so very low it looks as though maize does not cause any marked change in soil nitrates in the field. In the immediate vicinity of large clumps of roots and stubble this statement would not hold.

Carbon dioxide accumulation. The amounts of carbon dioxide evolved from soils treated with the various residues were determined over a period of 28 days. In preparing the cultures for this work residues were added on an equal nitrogen basis. Only one amount of each residue was used. The low clover residue amount (0.2 gm. per 100 parts of soil) was taken as the standard.

Immediately after the experiment was set up large amounts of carbon dioxide were evolved (fig. 1). This was especially noticeable in the case of the timothy

residue treatment but was not so pronounced in the check soil. After the fourth day the differences per period for any one treatment were not so marked. Toward the end of the period all of the curves show a tendency to flatten out. This is less true of the timothy treatment curve than of the others. The carbon dioxide curve of the clover residue treatment never exceeded, or even approached, the treatment of the timothy residues. This would indicate that there was no sudden falling off of the activities of microörganisms, as one would

TABLE 7

Effect of varying amounts of maize roots (no stubble) on the accumulation of nitrates in the soil

NUMBER	TREATMENT PER 100 GM. OF DRY SOIL	NITRATES EXPRESSED IN PARTS PER MILLION OF DRY SOIL AFTER				
		2 weeks	6 weeks	12 weeks	18 weeks	24 weeks
1	No treatment	90.21	123.11	231.06	364.16	300
2	0.2 gm. of corn roots	90.33	141.57	245.33	282.44	268
3	0.4 gm. of corn roots	65.35	112	182.50	389.33	324.5
4	0.6 gm. of corn roots	61.30	120.2	219.16	417	291.83
5	0.8 gm. of corn roots	46.82	98.5	187	396	262.66
6	1.0 gm. of corn roots	37.76	107.50	179.33	376.33	282.16
7	2.0 gm. of corn roots	23.41	39.81	315.83	366	280.16
8	3.0 gm. of corn roots	5.98	11.03	200.16	402.66	238.66

TABLE 8

*Effect of dried blood on the accumulation of nitrates in the soil**

NUMBER	NITRATES EXPRESSED IN PARTS PER MILLION OF DRY SOIL AFTER				
	2 weeks	6 weeks	12 weeks	18 weeks	24 weeks
1	90.21	123.11	231.06	364.16	300
2	69.55	180.33	212.5	474.51	310.66
3	92.25	198.44	301.16	475.66	271.83
4	97.57	230.22	323.33	528.66	208.16
5	100.23	273.08	418.83	573.83	332.66
6	110.66	335.5	444.50	643.50	386.5
7	107.83	440.28	732.33	736.5	883.33
8	128.21	574.09	765.6	978.5	1127.33

* Treatment added dried blood to furnish same amounts of N as the corresponding treatments of corn roots in table 7.

infer from the bacteria counts. It would point to the conclusion that organisms other than bacteria shown on the plates were active.[5] These must be important in assimilating soluble nitrogen. In fact these "other" organisms must have been largely responsible for the major portion of the evolution of carbon dioxide and accumulation of nitrogen after the first few days.

[5] Total counts of bacteria by the plate method were included in this study. These results have been omitted in order to save space.

The figures show that the maize residue treatment evolved less carbon dioxide up to and including the tenth day than did the clover residues. The difference between these two materials is further shown from the different effects on nitrate depression. Where nearly equal amounts of the two materials are used the immediate depression is greater from clover residue treatments. This is rather surprising since the carbon-nitrogen ratio of maize is considerably wider than that of the clover. That there is a difference in availability of nitrogen or energy material or both is apparent. Differences in carbon-nitrogen ratios of different material do not appear to tell the entire story.

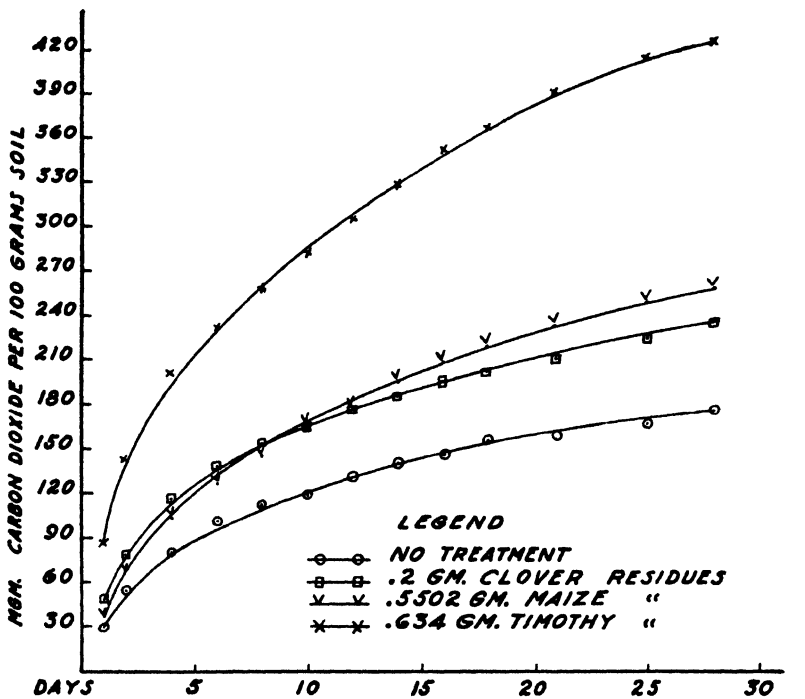

FIG. 1. TOTAL AMOUNTS OF CO_2 EVOLVED FROM SOIL TREATED WITH EQUAL QUANTITIES OF NITROGEN IN DIFFERENT CROP RESIDUES

Nitrification of water-soluble extracts of residue. Equal weights of timothy and clover residues, ground to pass a coarse screen (2 mm.) were soaked in distilled water (30 parts of water to 1 of residue) for 2 days. At the termination of this time the extracts were filtered and applied to soil in tumblers. Each 100 gm. of soil received the leachings from 0.4 gm. of residue.

The results of these studies are shown in table 9. Both extracts caused a depression of nitrates. The depression was more marked in the treatment receiving timothy extract than in the clover extract treatment.

Qualitative tests for vanillin in the extracts from the residues of clover,

maize, and timothy failed to show the presence of this toxic material. Collison and Conn (4) found this and other toxic organic compounds in extracts from straw. They were of the opinion that the immediate ill results that often followed applications of straw were due in part to a chemical toxic substance.

Series 2

The purpose of this study was to compare the effects of the additions of clover and timothy residues on soil nitrate changes, when they were applied on an equal carbon basis. The basic figure used for this study was 0.4 gm. of timothy residue per 100 gm. of soil. This amount was chosen since it was a little below

TABLE 9

Soil nitrate changes induced by additions of water extracts from crop residues

Results expressed as variation from untreated soil

INCUBATION PERIOD	NITRATES IN P.P.M. DRY SOIL	
	Timothy	Clover
days		
1	−4.1	1.9
2	4.6	2.0
4	−9.2	9.6
7	−57.0	−14.4
10	−15.8	−11.2
14	−43.8	−5.2
21	6.5	45.6
28	−57.4	−30.0
35	−44.7	−3.0
42	−36.0	−3.3
49	−14.0	−29
56	−26.0	19
70	. . .	. . . . .
84	−29.3	4.0
105	−37.0	1
133	−24.0	−14.0
210	60.0	66.0

the lowest amount of this residue found in the field. The same amount of carbon was supplied by 0.45 as by 0.4 gm. of timothy. Figure 2 shows the results of this work. The clover residues caused just as marked a depression of nitrates during the first 18 days as did the timothy. After 18 days, nitrates began to accumulate in the clover residue treatments more rapidly than in the other treatment. From shortly after the twenty-seventh day until the end of the experiment there was very little difference in nitrate contents between the untreated and clover residue treated soils. The timothy residues continued to show a depression of nitrates during the entire 60 days.

A study of the rates and total amounts of carbon dioxide evolved from soils

treated as in the nitrate work was made. The total evolution of carbon dioxide was much greater from the clover treatments than from the timothy (table 10). The greatest difference between the two treatments occurred within the first two weeks. The latter part of the sixty day run found the timothy treatment evolving only slightly more carbon dioxide than the clover.

It is interesting to note that the greatest depression of nitrates in the case of the clover residue treatment occurred at the time of the greatest carbon dioxide

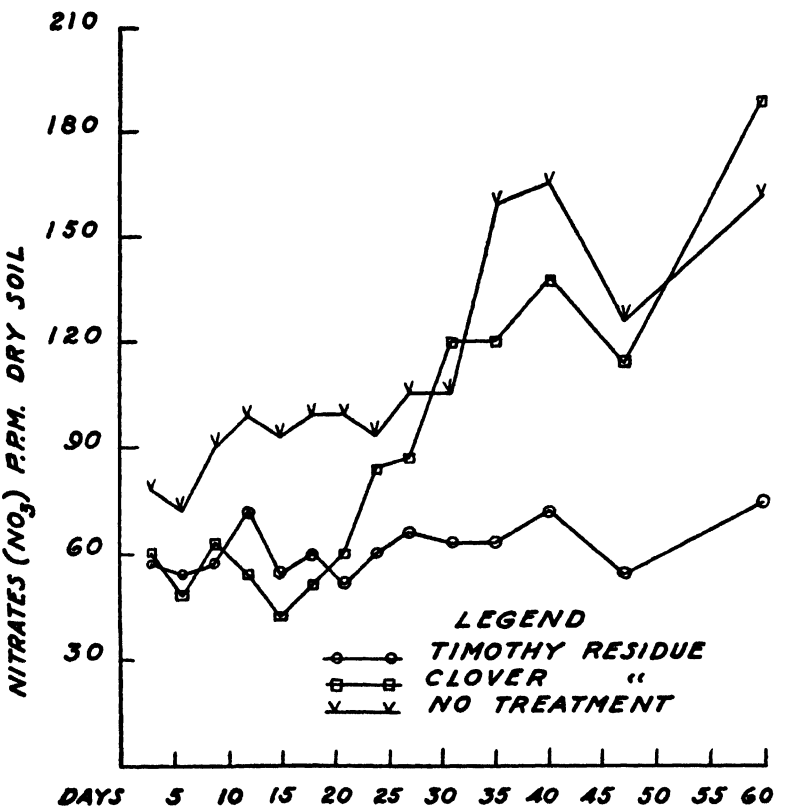

FIG. 2. EFFECT OF PLANT RESIDUES ON CHANGES IN SOIL NITRATES WHEN ADDED IN EQUAL CARBON AMOUNTS

evolution. There are no pronounced breaks in either the carbon dioxide or the nitrate curves of the timothy treatment.

The two materials having been applied on an equal carbon basis the changes in nitrates and carbon dioxide evolution must be due to factors other thon differences in total organic carbon contents. The total analyses show that there is a much narrower carbon-nitrogen ratio in the case of the clover residues than in the timothy residue. It would appear then that the lack of nitrogen prevented a more rapid evolution of carbon dioxide from the timothy residue.

This is another way of saying that biological activity was limited in the case of the timothy residue by lack of nitrogen. Heck (8) states:

> A given amount of energy material when used by soil organisms requires a given amount of nitrogen for decomposition and this nitrogen is built up into organic form in the microbial substance. Any nitrogen in excess of this amount is liberated in the mineral form.

Series 3

The carbon-nitrogen ratios of clover and timothy residues were made the same in another set of soil cultures. This was done by adding sufficient nitrogen in the form of nitrate of soda to the timothy residue in the series 2 treatment. A check treatment consisting of soil plus a similar quantity of nitrate of soda was set up at the same time. The effect of this treatment on nitrate depression and carbon dioxide evolution is given in figure 3 and table 11.

TABLE 10

Amounts of carbon dioxide evolved from soils treated with timothy and clover residues applied on an equal carbon basis

ASPIRATION PERIODS	MGM CARBON DIOXIDE PER 100 GM. OF SOIL (MOISTURE-FREE)		
	Check (no treatment)	Variation from check due to addition of timothy residues	Variation from check due to addition of clover residues
days			
3-day periods			
0–3	6.572	16.583	22.578
3–6	8.772	13.475	26.648
6–9	9.306	12.172	25.184
9–12	10.26	12.10	14.22
4-day periods			
12–16	11.33	9.74	12.10
16–20	10.56	12.82	11.97
20–24	8.8	10.51	11.23

The addition of sodium nitrate to the soil alone did not materially effect the biological activities of the soil. At least the rate and amounts of carbon dioxide evolved from the soil with sodium nitrate and the one without were very nearly the same. The addition of nitrogen caused an increase in the evolution of carbon dioxide when added to the timothy treatment. A comparison of the carbon dioxide accumulation of the clover residue treatment with that of the nitrate of soda timothy residue treatment show the two sets of figures to be very similar. The clover treatment evolved more total carbon dioxide than did the timothy plus sodium nitrate treatment. The greater difference came within the first 2 weeks. After the first 2 weeks the two treatments produced about the same amounts of carbon dioxide for some time. The last few weeks of the 60-day experiment, the rate of evolution of carbon dioxide from the timothy plus nitrogen exceeded that of the clover treatment.

Timothy residues plus sodium nitrate caused a very marked disappearance of nitrates over soil plus sodium nitrate. In comparison to the clover treatment the timothy plus sodium nitrate at all times contained more nitrates than did the clover treatment.

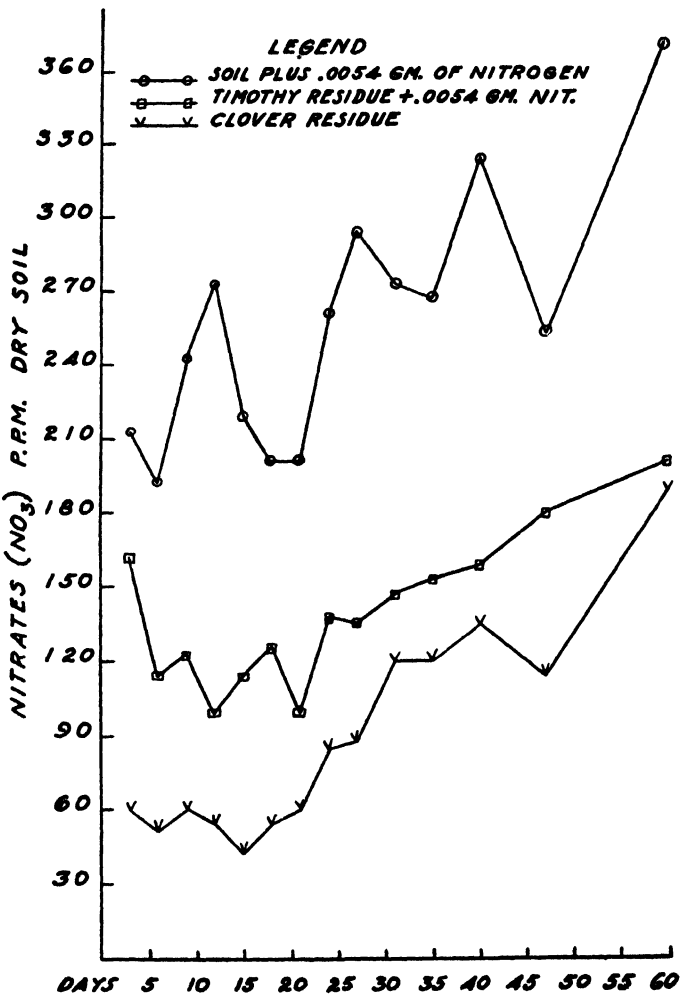

FIG. 3. EFFECT OF ADDITION OF PLANT RESIDUES ON ACCUMULATION OF SOIL NITRATES

The applications of residues were made on an equal carbon basis. Nitrate of soda was added to the timothy residue in amounts sufficient to make the C/N ratios of the treatments the same.

From a review of the results of both types of experiments used in series 2 and series 3 it is evident that there is a very marked difference in nitrates and in carbon dioxide evolved from soils treated with equal carbon amounts of

timothy and clover residues. These differences are not so noticeable where the carbon nitrogen ratios of the two materials have been made the same by addition of sodium nitrate to the timothy treatment.

TABLE 11

Amounts of carbon dioxide evolved from soil treated with timothy and clover residues

Carbon-nitrogen ratios were made equal by addition of sodium nitrate with the timothy residue.

ASPIRATION PERIODS	MGM. OF CARBON DIOXIDE EVOLVED PER 100 GM. DRY SOIL		
	Check (nitrate of soda only)	Variations from check due to additions of timothy residue and nitrate of soda	Variation from check due to addition of clover residues
days			
3-day periods			
0–3	5.475	20.432	23.675
3–6	8.882	21.89	26.538
6–9	11.99	15.18	22.50
9–12	8.25	17.88	16.23
4-day periods			
12–16	10.45	13.95	12.98
16–20	9.27	14.21	13.26
20–24	10.93	8.59	8.10

TABLE 12

General analysis of roots and stubble of timothy, clover, and corn on a moisture-free basis

	PER CENT IN 4 GM. OF FINELY GROUND MATERIAL		
	Timothy residue	Clover residues	Corn residues
Ether extract	0.35	0.71	0.79
Organic matter soluble in cold water	23.51	17.83	8.67
Lignin (ash-free, protein free)	32.11	35.32	38.41
Protein insoluble in cold water	2.32	7.00	1.54
Ash	6.89	13.54	19.23*
Cellulose and pentosans†	34.82	26.30	31.36
Total	100.00	100.00	100.00
Soluble nitrogen	.154	.545	.265
Part of total nitrogen soluble	30.3	34.8	43.65

* The high ash content must be due in part to adhering particles of soil which could not be washed off.

† Obtained by difference.

General analyses of residues

General analyses of clover, corn, and timothy residues were made. The methods were similar to those used by Waksman and Tenny (36). The results

of these analyses (table 12) show many differences between the three residues which might be responsible for the differences observed in the evolution of carbon dioxide and nitrate changes when these three materials were applied to the soil.

Among the many differences shown by these analyses perhaps the most striking and important are the figures which show the cold-water soluble nitrogen and organic matter. Wilson and Wilson (42) worked with roots of sorghum and maize on an equal weight basis. They noticed a greater immediate depression of nitrates following an application of sorghum roots than following maize roots. They attributed this difference to the greater amount of soluble energy applied by the sorghum roots. Whiting and Richmond (40) believe that the largest single factor in the initial rate of decomposition is the amount of water-soluble nitrogen.

It would seem that the immediate effect on the change in nitrate concentration in the soil solution upon the addition of residues can best be explained upon differences of both soluble nitrogen and soluble organic matter. The C : N ratios of the water-soluble portions of these residues are approximately; corn 5:1, clover 10:1, and timothy 88:1.

That water extracts from the two residues (clover and timothy) cause a difference in nitrate depression has been shown. The water extract applied to each 100 gm. of soil was obtained from 0.4 gm. of residue. In series 2 the amount of timothy residue applied was 0.4 gm. It is possible therefore to compare the effect of the extract and the material in this case. After the first 2 weeks the nitrate depression caused by the timothy residue added directly was more severe than that caused by the timothy extract. Up to the first 2 weeks there seemed to be little difference between the two treatments. A considerable proportion of the nitrate depression certainly seems to be caused by the soluble organic material. The later depression must be attributed in part to the insoluble constituents.

The literature review brought out the fact that cellulose and other relatively insoluble organic compounds caused a depression of soil nitrates. Waksman and Tenny (37) and Rege (26) have shown that lignin is a fairly resistant material, which does not decompose rapidly in the soil. Waksman and Tenny base this conclusion on the fact that in the "so-called soil humus and humic acid" portion of the soil, lignin occupies a leading rôle. They designate the organic matter remaining after subtracting the sum of the lignin and ether-soluble extracts from the total organic matter as "available energy material." The analyses of the residues show that timothy contains the largest proportion of available energy material from this viewpoint. Rege's work shows that the presence of a large percentage of lignin is detrimental to the decomposition of cellulosic matter. It seems to act as an inhibiting factor; perhaps it acts as a physical barrier.

SUMMARY

A study was made to determine whether the amounts of roots and stubble of red clover, maize, and timothy present at harvest time are sufficient to cause a marked change in the nitrate content of soil.

The amounts of roots and stubble left in the soil, at harvest time, were determined. The effects of additions of various amounts of crop residues to soil were determined both upon changes in soil nitrate and upon the evolution of carbon dioxide. General plant analyses were also made.

The amount of dry matter in the soil in the form of red clover roots and stubble amounts to only about half the weight of timothy roots and stubble from equally good stands. The percentage of dry matter in the soil in the form of corn roots and stubble is exceedingly small in comparison with that of the other two crops.

The actual weight of roots of these crops found below 7 inches was relatively unimportant. The soils from which these crops were taken are characterized by fairly heavy subsoils, which probably accounts for the meager root development in the lower horizons.

The nitrification studies show that field amounts of clover and timothy are sufficient to influence the accumulation of soil nitrates. All amounts of timothy residues used resulted in a depressing effect of nitrates over the no treatment. The larger the amounts of timothy residues added, the longer was the depression period severe. Clover residues seemed to cause a temporary depression of soil nitrates during the first period of incubation.

Nitrification studies where corn residues were added to the soil showed that before soil nitrates were affected by addition of this material a much larger amount had to be added than is found in the field except in the immediate vicinity of large clumps of roots and stubble.

Carbon dioxide curves show that high carbon dioxide evolution is correlated rather closely with nitrate depression.

Water extracts from timothy residues, produced, when mixed with soil, a greater depression of soil nitrates than did another extract from clover residues.

The carbon-nitrogen ratios of clover and timothy residues were made the same in another set of soil cultures. This was done by adding the residues on an equal carbon basis and supplementing the timothy treated cultures with nitrogen in the form of sodium nitrate.

Timothy residues plus sodium nitrate caused a very marked disappearance of nitrates over soil plus sodium nitrate. In comparison to the clover treatment the timothy plus sodium nitrate at all times contained more nitrates. The addition of sodium nitrate to the soil did not produce any marked change in carbon dioxide evolved. The carbon dioxide evolution was greatly increased by the addition of sodium nitrate to the cultures receiving the timothy residues.

General analyses show a number of interesting facts. Timothy residues which caused the greatest depression of soil nitrates contained the highest per-

centage of organic matter soluble in cold water. The timothy residues also contained the largest amount of so-called available energy material and the least amount of water-soluble nitrogen of the three residues. Corn, which caused the least depression of soil nitrates, contained the smallest percentage of organic matter soluble in cold water and the least amount of available energy material. It did not contain the highest percentage of water-soluble nitrogen, but the carbon-nitrogen ratio of the water-soluble portions was narrowest in corn of the three residues.

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THE EFFECT OF CULTURE SOLUTIONS ON GROWTH AND NITROGEN FRACTIONS OF OAT PLANTS AT DIFFERENT STAGES OF THEIR DEVELOPMENT[1]

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In recent years culture solutions have been commonly employed in studying the nutritional needs of economic plants and the influence of various elements upon plant growth. A knowledge of the chemical nature of the media in which plants are grown, such as is furnished by these synthetic cultures, has been of primary value in the solution of many nutritional problems.

Many investigations have been carried out which deal with the influence of the several elements required by plants in major quantities, their proportions, and concentrations in culture media, upon growth and yields. It is now generally conceded that the range of salt proportions in a culture solution favorable to plant growth is relatively wide. Hoagland (4) states "there is not sufficient evidence that plants require for optimum yields any very specific ratio of elements within wide limits, providing the total supply and concentration of the essential elements are adequate." But, under the usual cultural methods, total supply is noticeably limited, and concentrations of some of the essential elements change rapidly unless some adequate system of continuous flow for the constant renewal of these elements is employed. It is, however, possible, as will be brought out and as has previously been suggested, that plants of different ages may require different proportions of the essential elements for optimum growth.

That the composition of plants varies with the composition of the solution in which they are grown, has been known since the days of de Saussure (7). Hoagland (4), Dickson (1), and others (2, 3) have shown that with an increase of an element in the solution there is a corresponding increase of that element within the plant; but how the more complex organic materials such as the carbohydrates, fats, and proteins are affected by the ionic relations of the solution in which the plants are grown, has not as yet been thoroughly investigated.

The primary purpose of the present work was to investigate the quantitative relationships between the essential inorganic constituents of a series of culture solutions of known composition and the nitrogenous nature and quantity of

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plant tissue which these solutions are capable of producing. It involves a quantitative study of various nitrogen fractions of oat plants and how these fractions are influenced by the salt proportions, particularly with respect to the proportions of the nitrogen bearing salts, of the solutions in which they are grown, at different stages of development throughout the life cycle.

PLAN OF EXPERIMENTS

Three series of oat plants, designated as A, B, and C, were grown in culture solutions. Each series included 20 individual cultures, and all solutions differed in their salt proportions in accordance with a definite plan. The corresponding solutions of the three series were identical, and all cultures were similarly treated to every respect except that the plants from the three series were harvested when 20, 40, and 60 days old, respectively.

At the time of harvest the plants of series A were in the early seedling stage, having an average height of 28 cm. Those of series B were in the early vegetative stage and heads were just beginning to form in the sheaths. The height of the plants at this harvest varied somewhat, but averaged about 50 cm. When the plants of series C were harvested they were well past the heading stage. The size of the plants at this age varied greatly, depending upon the salt proportions of the solutions in which they were grown. Some plants in the poorly balanced solutions failed to produce panicles.

The plants were grown in the greenhouse during the summer months—June, July, and August. The temperature averaged about 25°C. and there was an average daylight period of approximately 15 hours.

Description of solutions

The 20 culture solutions employed were chosen from the Tottingham complete series of 84, and were modified as described by Jones and Shive (5). This modification consisted of the substitution of $(NH_4)_2SO_4$ for KNO_3 in equivalent partial osmotic concentrations, and each solution when complete possessed a total osmotic concentration of one atmosphere. The solutions chosen were uniformly distributed throughout the Tottingham series and will be designated by the culture numbers referring to the position which they occupy in the series and on the four-coördinate diagrammatic scheme employed by Tottingham, as well as by the values of their numerical succession from 1 to 20. The solution cultures were prepared from half-molecular stock solutions of the salts employed. The partial volume-molecular concentrations of the salts as they occurred in the different solutions, the solution numbers indicating their position in the modified Tottingham series (5) are given in table 1.

In order to avoid any pronounced change in either concentration or salt proportions of the solutions through the action of the growing plants, the solutions were continuously renewed at constant rates by the method described by Shive and Stahl (10). Two and one-half liters of fresh solution were passed through each culture jar during a 24-hour interval.

Iron was added to the cultures in the form of ferrous sulfate. A fresh 0.1 per cent solution was prepared each day and added in amounts which varied according to the needs of the plants. An average of about 1 cc. of this solution per liter of culture solution was employed. Much less than this was use during the seedling stage and proportionately more during the vegetative and reproductive phases.

Cultures and material employed

Seven and one-half liter glazed stone crocks were used as culture receptacles. A tightly fitting circular lid was made for each crock from beaver-board and

TABLE 1

Description of culture solutions

SOLUTION NUMBER	CULTURE SOLUTION DESIGNATION	PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			
		KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	$MgSO_4$
1	$T_1R_1C_1$	0 00211	0 0014	0 00146	0.01659
2	$T_1R_1C_3$	0 00211	0 0014	0 00438	0 01185
3	$T_1R_1C_5$	0 00211	0 0014	0 00730	0 00711
4	$T_1R_1C_7$	0 00211	0 0014	0 01022	0 00237
5	$T_1R_3C_1$	0 00211	0 0042	0 00146	0 01185
6	$T_1R_3C_3$	0 00211	0.0042	0.00438	0.00711
7	$T_1R_3C_5$	0 00211	0 0042	0 00730	0 00237
8	$T_1R_5C_1$	0 00211	0 0070	0 00146	0 00711
9	$T_1R_5C_3$	0 00211	0 0070	0 00438	0 00237
10	$T_1R_7C_1$	0 00211	0 0098	0 00146	0 00237
11	$T_3R_1C_1$	0 00633	0 0014	0 00146	0 01185
12	$T_3R_1C_3$	0 00633	0 0014	0 00438	0 00711
13	$T_3R_1C_5$	0 00633	0 0014	0 00730	0 00237
14	$T_3R_3C_1$	0.00633	0 0042	0 00146	0 00711
15	$T_3R_3C_3$	0 00633	0 0042	0 00438	0 00237
16	$T_3R_5C_1$	0 00633	0 0070	0.00146	0 00237
17	$T_5R_1C_1$	0 01065	0 0014	0 00146	0 00711
18	$T_5R_1C_3$	0 01065	0 0014	0 00438	0 00237
19	$T_5R_3C_1$	0 01065	0 0042	0 00146	0 00237
20	$T_7R_1C_1$	0 01477	0 0014	0 00146	0 00237

heavy waxed paper. The heavy paper was cut into strips 5 cm. wide and sufficiently long to encircle the crock. The strip was folded longitudinally so that the folded halves stood at a right angle to each other. Successive cuts were then made at intervals of about 1.5 cm. from one outer edge to the center of the horizontal strip so as to allow the cut edges to overlap each other when the strip was brought into a circular form around the crock. The cut side was then sewed firmly to the lower surface of the circular board lid in such a manner that the uncut side formed a flange around the cover which served to hold the cover firmly in position on the crock. Six holes, each 5 cm. in diameter, were bored in the lid and a tightly fitting cork stopper was placed in each hole. The entire lid was then dipped in melted paraffin.

A pure line of oats (Burt Nebraska 4) was used. Healthy seeds of the same general size were chosen and germinated between blotting paper. When the primary roots were 3 mm. long, selection of similar seedlings was made and they were transferred to a germinating net such as was used by Shive (9). The root tips were inserted in a solution having an osmotic concentration of 0.5 atmosphere and the following salt porportions: KH_2PO_4, 0.00211; $(NH_4)_2SO_4$, 0.00420; $Ca(NO_3)_2$, 0.00730; and $MgSO_4$, 0.00237 moles per liter. After a few days the seedlings were carefully selected for uniformity as to height (5–6 cm.) and general appearance, placed in the cork stoppers according to the method employed by Tottingham (11), and transferred to the culture jars. In series B and C 3 plants were placed in each cork making a total of 18 plants per culture, but in order to obtain sufficient green tissue for analysis for series A, the plants of which were harvested when only 3 weeks old, it was necessary to place 6 plants in each cork stopper making a total of 36 plants to each culture.

ANALYTICAL PROCEDURE

Handling of tissue. Only the tops of the oat plants were used. They were severed from the roots and lower stems 2.5 cm. above the cork stopper and about 3.5 cm. above the uppermost root. As the plants from each culture were harvested their green weight was immediately determined. The tissue was then rapidly cut into sections about $\frac{1}{2}$ mm. long and these were thoroughly mixed. From this minced material duplicate samples were taken, accurately weighed, and dried for moisture determinations, while the bulk of the tissue was immediately weighed and used for aqueous extractions of the nitrogen fractions.

Extraction and determination of the nitrogen fractions. In obtaining the plant extract, the fresh tissue was ground in a large mortar with nitrogen-free quartz sand. Water was added to this material and the whole was transferred to a large funnel over which was placed an 18-inch square of lawn cloth. The extract was forced through the cloth by moderate wringing by hand. The resulting extract was heated to boiling, 3 cc. of 10 per cent acetic acid was added, and boiling was continued for 3 or 4 minutes. The coagulum formed was removed from the solution by filtering through a Büchner funnel into which were placed a filter paper and some nitrogen-free paper pulp. The coagulum-free filtrate was reduced in volume to 500 cc. by gently boiling, and 100-cc. aliquots were taken for analysis. It was found that by this procedure the non-coagulable extracted nitrogen was completely removed, and additional grinding and further extraction gave no increase of nitrogen in this extract.

Ammonia and nitrate nitrogen. These determinations were made on 100-cc. aliquots of the coagulum-free extract, using a method (8) by means of which the two fractions, ammonia and nitrate nitrogen, can be determined on the same sample with a high degree of accuracy. Any nitrite nitrogen present is included as nitrate nitrogen.

Inorganic nitrogen. This fraction is obtained by adding together the nitrogen from the ammonia and nitrate determinations.

Soluble organic nitrogen. Since all forms of inorganic nitrogen found in plant tissues are considered to be soluble, the soluble organic nitrogen was obtained by subtracting the total inorganic from the total soluble nitrogen.

Insoluble organic nitrogen. This fraction is often designated as "protein nitrogen," but since it is obtained by subtracting the soluble nitrogen from the total nitrogen, it includes not only the protein nitrogen but the insoluble nitrogen from other substances, such as glucosides, nitrogenous lipoids, plant bases, and nucleic acids.

Total organic nitrogen. This includes all the organic nitrogen forms, soluble and insoluble, and is obtained by subtracting the total inorganic nitrogen from the total nitrogen of the plant.

Total nitrogen. This was determined by Kjeldahlization of some of the tissue previously used for moisture determinations. The determinations were made according to the directions given by Ranker (6), care being taken to adhere to his suggestions in every detail.

EXPERIMENTAL RESULTS

Growth relations and dry weights

The dry weight of the plants produced by the individual cultures was determined in the usual way. Some interesting relationships exist between the dry weights of the plants at the different stages of development and the chemical composition of the solutions in which they were grown, as will be brought out in the consideration of the yields from the three series of plants here described.

The numerical data representing the yield values for each of the three series are presented in table 2, together with the relative osmotic proportions of the salts in the various solutions. For the sake of convenience in comparing the effect on yields shown by differences in the osmotic proportions of the nitrogen bearing salts in the culture solutions, it will be observed that the cultures and the data obtained from them are grouped in pairs. The grouping is such that, except for the last three pairs, the osmotic values of $MgSO_4$ in the two solutions of each pair are the same, as are also the osmotic values of KH_2PO_4. It will be observed, however, that while the sums of the osmotic concentrations from the two introgen bearing salts are equal in the two solutions of each pair, the concentration of the ammonium salt in the first solution of each pair is low and the nitrate salt is high, while in the second solution of each pair the ammonium salt is correspondingly high and the nitrate salt correspondingly low. Thus, in the third pair of cultures (numbers 18 and 19), $MgSO_4$ has an osmotic value of one-tenth of the total osmotic concentration of one atmosphere in each solution, and KH_2PO_4 in each solution has an osmotic value of five-tenths of the total osmotic concentration of the solution. The osmotic concentration of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ in the first solution of the pair is one-tenth and three-tenths and in the second solution three-tenths and one tenth, respectively,

of the total osmotic concentration of the solution. In the last three pairs of cultures in the table, the nitrogen bearing salts show the same osmotic values in each pair. This arrangement greatly facilitates comparisons with respect to the effect on yields of the differences in the osmotic values of the two nitrogen bearing salts. All the succeeding tables are constructed in a similar

TABLE 2

Influence of ammonium and nitrate nitrogen in the culture solutions on the growth of oat plants of different ages

PAIRS OF CULTURES	SOLUTION NUMBER		OSMOTIC PROPORTIONS				DRY WEIGHT OF 20 PLANTS		
			$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A, plants 20 days old	Series B, plants 40 days old	Series C, plants 60 days old
							gm.	*gm.*	*gm.*
1	12	$T_3R_1C_3$	3	3	1	3	**1.70**	**11.80**	**25.75**
	14	$T_3R_3C_1$	3	3	3	1	1.45	5.80	8.83
2	2	$T_1R_1C_3$	5	1	1	3	1 22	**10.56**	24.06
	5	$T_1R_3C_1$	5	1	3	1	1.15	3.90	8.37
3	18	$T_5R_1C_3$	1	5	1	3	1 14	**10.55**	**24.22**
	19	$T_5R_3C_1$	1	5	3	1	1.27	10.24	14.15
4	3	$T_1R_1C_5$	3	1	1	5	1 20	9.31	**26.50**
	8	$T_1R_5C_1$	3	1	5	1	1.29	5.72	8.08
5	13	$T_3R_1C_5$	1	3	1	5	1 39	10 07	16.72
	16	$T_3R_5C_1$	1	3	5	1	1 21	8.55	11.22
6	4	$T_1R_1C_7$	1	1	1	7	1.21	7.34	**24.58**
	10	$T_1R_7C_1$	1	1	7	1	1 30	6.17	Killed
7	7	$T_1R_3C_5$	1	1	3	5	**1.42**	9 34	17.20
	9	$T_1R_5C_3$	1	1	5	3	**1.54**	9.00	14.62
8	20	$T_7R_1C_1$	1	7	1	1	1.28	7 40	20.01
	1	$T_1R_1C_1$	7	1	1	1	0 80	4.59	7.42
9	17	$T_5R_1C_1$	3	5	1	1	1.13	8.12	22.71
	11	$T_3R_1C_1$	5	3	1	1	1.23	5.77	8.86
10	15	$T_3R_3C_3$	1	3	3	3	**1.70**	**10.56**	**27.80**
	6	$T_1R_3C_3$	3	1	3	3	1.18	**10.66**	23.90

manner. The dry weights of the cultures producing the highest five yields in each series appear in bold-face type.

From an examination of the yield values of the younger and older plants as presented in table 2, it will be observed that solutions favorable to good growth of the younger plants (series A) do not always correspond with those producing

most favorable growth in the older plants of series B and C. The five solutions producing high yields in the series of young plants (series A) are numbers 7, 9, 12, 14, and 15; whereas in the series of the older plants (series C) they are solutions number 3, 4, 12, 15, and 18. The pronounced differences in yields between solution 20 and 1, and also 17 and 11 of pairs 8 and 9 respectively, in series B and C may not be due so much to the nitrogen bearing salts, which are very low in these cultures, as to the general unbalanced condition of the salt proportions in these cultures.

Thus when the plants are in the early stages of development, there is no very pronounced difference between the yields from the high ammonia and high nitrate cultures. In four out of the seven pairs of cultures in which the nitrogen bearing salts show differences in their osmotic values, the larger yields for series A are slightly in favor of the high ammonia cultures. This condition, however, is strongly reversed in the older plants (series B and C). In every case the solutions relatively high in ammonium nitrogen and low in nitrate nitrogen greatly retarded the growth of the older plants, whereas the high yields were produced by the cultures relatively low in ammonium nitrogen and relatively high in nitrate nitrogen. This clearly brings out the fact that from the standpoint of dry weight production the young plants are just as efficient in the utilization of ammonium nitrogen as they are in the use of nitrate nitrogen, whereas the older plants utilize nitrate nitrogen much more effectively than they do ammonium nitrogen. Some interesting relations between these yield values and the nitrogenous composition of the plants from the various solutions will be presented.

It is clear from the data of table 2 that in the utilization of nitrogen as ammonium, the young plants are much more efficient than are the older plants, whereas in the use of nitrate nitrogen the older plants are much more efficient than the young plants, when measured by the criterion of dry plant material produced.

Presentation of analytical data

Nitrogen as ammonia. The numerical data representing the results of the analysis for the inorganic nigrogen fractions in the plants of series A (20 days), B (40 days), and C (60 days) are presented in table 3. The data are given in percentages of nitrogen as ammonia and as nitrate on the dry weight basis. The cultures, together with the corresponding analytical data, are arranged in pairs in the same order as in table 2.

Although the nitrogen as ammonia in the plants of the three series is low and represents but a small fraction of the total inorganic nitrogen in the plants, yet this fraction increases or decreases, but not in direct proportion, with increase or decrease respectively of nitrogen as ammonium in the solutions in which the plants were grown. It will be observed that three cultures high in nitrogen as ammonia in each series are corresponding cultures. All the high yielding cultures in each of the three series are associated with a high relative

proportion of ammonium sulfate in the solutions in which the plants were grown, and this relation is the same in the young plants as it is in those of intermediate age and in the mature plants. On the other hand, low yields of nitrogen as ammonia are always associated with low relative proportions of

TABLE 3

Ammonia nitrogen, nitrate nitrogen, and total inorganic nitrogen in plants of series A, B, and C

Pairs of cultures	Culture solution number	Osmotic proportions				Nitrogen as ammonia			Nitrogen as nitrates			Inorganic nitrogen		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C	Series A	Series B	Series C	Series A	Series B	Series C
						per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	12	3	3	1	3	0 06	0 04	0 06	1.45	1.34	0.93	1.51	1.38	0.99
	14	3	3	3	1	0 09	0 10	**0.35**	1.73	0.95	1.20	1.82	1 05	**1.55**
2	2	5	1	1	3	0.06	0.04	0.06	1.87	**1.51**	1.21	1.93	**1.55**	1 27
	5	5	1	3	1	0 06	**0.15**	0 21	1.75	0.95	0.92	1.81	1.10	1.13
3	18	1	5	1	3	0 07	0 06	0 08	1 93	**1.80**	1.33	2.00	**1.86**	1 41
	19	1	5	3	1		0 11	**0.22**		1.31	1.26		1.42	1 48
4	3	3	1	1	5	0 05	0 06	0.06	**2.32**	**1.72**	1 25	**2.37**	**1.78**	1 31
	8	3	1	5	1	**0.14**	**0.17**	**0.40**	1 61	1 05	1.18	1 75	1.22	**1.56**
5	13	1	3	1	5	0 09	0 04	0 08	1 89	1 35	**1.35**	1 98	1.30	1 43
	16	1	3	5	1	**0.15**	**0.17**	**0.55**	1 73	1 06	0 68	1.89	1.22	1 23
6	4	1	1	1	7	0 06	0.05	0 06	**2.16**	**1.94**	**1.37**	**2.22**	**1.99**	1.43
	10	1	1	7	1	**0.20**	**0.32**	Killed	1.55	0.91	Killed	1.75	1.23	
7	7	1	1	3	5	0.09	0 08	0.15	**2.08**	**1.64**	**1.66**	**2.17**	**1.72**	**1.81**
	9	1	1	5	3	**0.13**	**0.18**	**0.28**	1.80	1.30	**1.74**	1.93	1 48	**2.02**
8	20	1	7	1	1	0.06	0 06	0 07	1 69	1.23	1.02	1.75	1.29	1.09
	1	7	1	1	1	0 07	0.07	0.08	1.57	1.07	1.06	1.64	1.14	1.14
9	17	3	5	1	1	0 06	0.06	0 07	1.77	1.42	0 96	1.83	1.48	1.03
	11	5	3	1	1	0 08	0 06	0.11	1 61	1.06	1.07	1.69	1.12	1.18
10	15	1	3	3	3	**0.10**	0.08	0.14	**2.03**	1.40	1.13	**2.13**	1.48	1.27
	6	3	1	3	3	0 09	0.08	0.11	**2.16**	1.31	**1.41**	**2.25**	1.39	**1.52**

ammonium sulfate in the solutions in which the plants were grown, regardless of the age of the plants.

To bring out the relation between nitrogen as ammonia in the plants of the various cultures and the age of the plants; that is, the effect of age on the accumulation of nitrogen as ammonia in the plants, the data of table 3 repre-

senting this fraction in series A were arranged in the descending order of their values. These data were plotted to form the graph of figure 1. The ordinates represent the percentage of nitrogen as ammonia, and the abscissas represent the different cultures of the series arranged in the descending order of the ammonia nitrogen values of the plants of series A. The corresponding data for the mature plants (series C) were then plotted, using the same abscissas and the same scale of ordinates.

From the graphs of figure 1, it is clear that although the percentage of nitrogen as ammonia is low throughout both series, the values for series C are always higher than are the corresponding values for series A. Although many values for corresponding cultures of the two series show only slight differences, several corresponding cultures of the two series show differences which are quite pronounced: as, for example, culture 16 ($T_3R_5C_1$) and culture 8 ($T_1R_5C_1$), or the values of the first cultures represented by the graph, in which the solu-

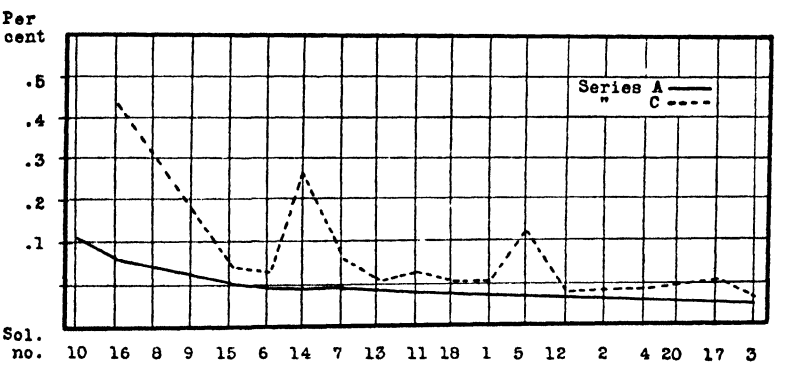

FIG. 1. GRAPHS SHOWING THE DIFFERENCE IN PERCENTAGES OF NITROGEN AS AMMONIA IN THE PLANTS OF CORRESPONDING CULTURES OF SERIES A (AT 20 DAYS) AND SERIES C (AT 60 DAYS)

tions were relatively high in ammonium sulfate, and also in cultures 14 ($T_3R_3C_1$) and 5 ($T_1R_5C_1$) in which a high proportion of the nitrogen in these solutions was present in the form of the ammonium salt.

It is to be noted as a matter of considerable interest, that nitrogen as ammonia is the only nitrogen fraction here considered which exhibits higher values for the older plants than for the younger plants of corresponding cultures. This accumulation of nitrogen as ammonia as the plants become older produced marked injurious effects in the plants grown in solutions with high proportions of ammonium sulfate, as has already been cearly brought out in connection with the discussion of dry weight yields. In every case, an increase of nitrogen as ammonia within the plants in the course of their development, as indicated in table 3, is directly correlated with decreased yields of dry plant material, as shown in table 2.

It appears that during the early stages of growth the plants are capable of

assimilating ammonium nitrogen as rapidly as they absorb it, even from solutions with relatively high concentrations of this form of nitrogen. As the plants become older, this power to assimilate ammonium nitrogen appears to diminish, under the conditions of these experiments, resulting in accumulation of ammonium nitrogen in the tissues, retardation of growth, and in some instances actual injury to the plants.

Nitrogen as nitrates. It will be observed from the data of table 3 that nitrate nitrogen comprises by far the greater portion of the inorganic nitrogen of these plants, and that the nitrate content of the plants of a given series varies with, but not in proportion to, the nitrate content of the solution in which the plants were grown. In general, cultures which produced plants high in nitrate nitrogen, regardless of age, are associated with high relative proportions of calcium

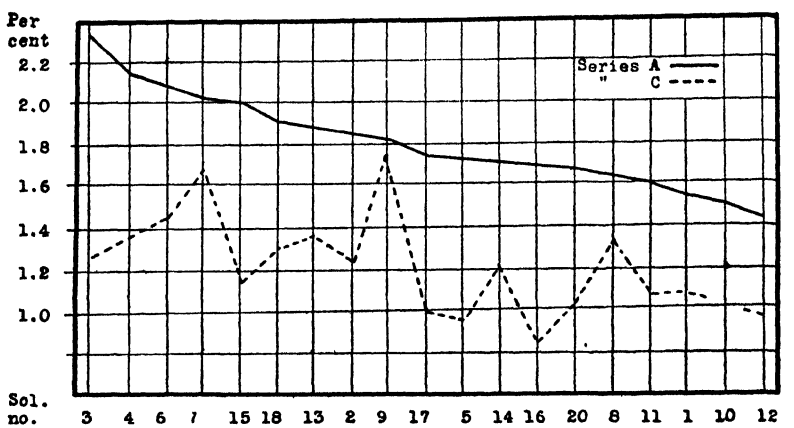

FIG. 2. GRAPHS SHOWING THE DIFFERENCES IN PERCENTAGES OF NITROGEN AS NITRATE IN THE PLANTS OF CORRESPONDING CULTURES OF SERIES A (AT 20 DAYS) AND SERIES C (AT 60 DAYS)

nitrate in the solutions in which the plants were grown; but this relation does not hold in all cases, but it is particularly pronounced in the cultures of series B.

Although the variation in the nitrate content of plants of the same age, resulting from differences in the chemical nature of the solutions with respect to nitrogen content, is not particularly pronounced except in series B, the variation in the nitrate content of plants of corresponding cultures at different ages is quite striking. The young plants of series A always show a higher nitrate content than do the older plants of series B and series C. This is clearly brought out by the graphs of figure 2. The data referring to nitrogen in the nitrate form found in the plants of series A as given in table 3 were arranged in the descending order of their values and plotted to form the upper graph of the figure. The corresponding values for nitrogen as nitrates in the plants of series C were then plotted in the same order, using the same scale of ordinates. The values of series B lie intermediate between those of series A and series C

and are not here shown. These graphs show clearly the marked differences in the nitrate values of the old and young plants of corresponding cultures. The marked decrease in this fraction as the plants increase in age is interesting, since, as previously shown, the ammonia nitrogen representing the other inorganic nitrogen fraction increased as the plants became older. The average

TABLE 4

Soluble organic nitrogen in plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBER	OSMOTIC PROPORTIONS				SOLUBLE ORGANIC NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C
						per cent	*per cent*	*per cent*
1	12	3	3	1	3	1 03	0.43	1.06
	14	3	3	3	1	0.76	0.10	1.30
2	2	5	1	1	3	0.71	0.63	0.71
	5	5	1	3	1	0.49	0.42	1.06
3	18	1	5	1	3	0.98	0.49	0.70
	19	1	5	3	1		0.74	0.95
4	3	3	1	1	5	0.89	0.60	0.71
	8	3	1	5	1	1.22	0.96	1.27
5	13	1	3	1	5	1 03	0 38	0 68
	16	1	3	5	1	1.16	0.60	1.97
6	4	1	1	1	7	1.02	0 53	0 89
	10	1	1	7	1	1.27	1.38	Killed
7	7	1	1	3	5	1.10	0.75	1.06
	9	1	1	5	3	1.27	1.11	1.60
8	20	1	7	1	1	0 90		0 58
	1	7	1	1	1	0.82	0 02	0.60
9	17	3	5	1	1	1.01	0.40	0.67
	11	5	3	1	1	0.96	0.20	0.56
10	15	1	3	3	3	1.21	0.65	1.09
	6	3	1	3	3	0.93	0.44	0.79

proportion of ammonia in the plants of series C is 79.3 per cent higher than the average in the plants of series A; while at the same time the average nitrate nitrogen in the plants of series C is 34.8 per cent less than this average in the plants of series A.

Soluble organic nitrogen. This fraction refers to the organic nitrogen found in the coagulum-free extract of the plants. Just what these soluble organic

nitrogenous compounds are is not known, but they are thought of as diffusible organic forms and building blocks for the more complex or conjugated protein. The percentage of nitrogen found in this form represents but a small fraction of the total organic nitrogen of the plants.

The data pertaining to the soluble organic nitrogen fractions for series A, B, and C are presented in table 4 in the same manner as were the data of previous tables. An examination of the data of this table brings out the fact that a relatively high content of soluble organic nitrogen in the plants is always associated with high proportions of nitrogen as ammonium in the solutions producing these plants, and that low yields of soluble organic nitrogen in each of the three series are definitely associated with relatively low proportions of nitrogen as ammonium in the culture solutions, regardless of the age of the plants. On the other hand, the data show that there is no such relation between the soluble organic nitrogen content of the plants and the nitrate nitrogen or the solutions in which they were grown.

A comparison of the data of table 3, relating to the ammonium nitrogen fraction, with those of table 4, representing the soluble organic nitrogen fraction of corresponding cultures of the three series, shows that cultures which produced high yields or low yields of one of these two fractions produced high yields or low yields, respectively, of the other fraction also.

As already pointed out, high and low values of these two fractions are definitely correlated with high and low proportions, respectively, of nitrogen as ammonium in the culture solutions. To emphasize these relations and to bring out more clearly the pronounced influence of the ammonium salt in the solutions upon the soluble organic nitrogen fraction and upon the content of nitrogen as ammonia in the plants, the values of these two fractions for the plants of series A, B, and C have been brought together and are diagrammatically presented in figure 3. On the left of the figure are given the percentage values of nitrogen as ammonia in the plants, and on the right the precentage values of the water-soluble organic nitrogen. At the base of the figure are represented in diagram the osmotic compositions of the nitrogen bearing salts, below which are given for each solution the osmotic values of the four salts in tenths of the total osmotic concentration of one atmosphere. The pairs of cultures are numbered to correspond with those given in the tables representing the values of the various nitrogen fractions.

These diagrams representing the three series of cultures show remarkable agreement between the nitrogen as ammonia and the total soluble organic nitrogen of the plants here considered. They bring out the fact that the values of these two nitrogen fractions of the plants are determined in large measure by the nitrogen as ammonium in the culture solutions: that is, in general, high proportions of nitrogen as ammonium in the culture solutions correspond to high yields of both soluble organic nitrogen and nitrogen as ammonia in the plants. With but one or two exceptions in series A and B, this relation is exact. On the other hand, the opposite relation exists with respect to these

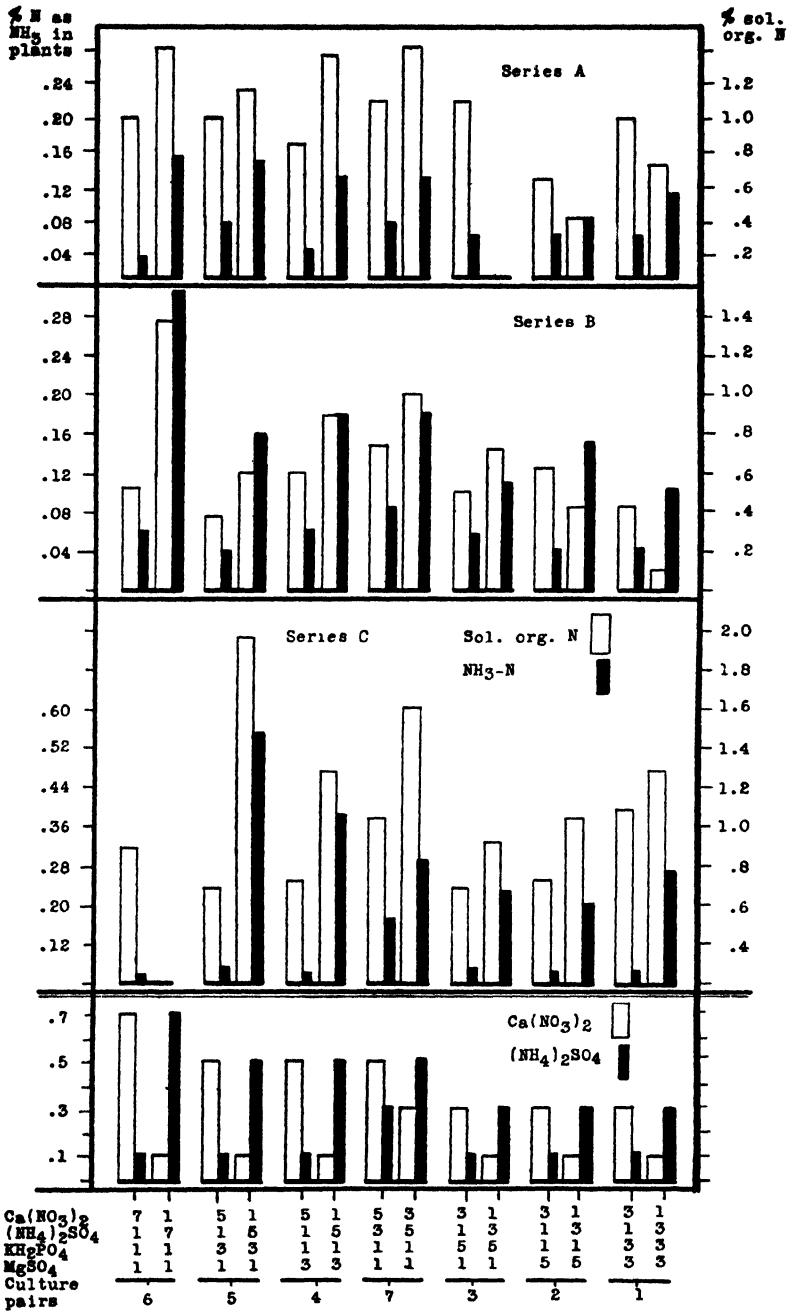

FIG. 3. DIAGRAM SHOWING THE RELATION BETWEEN THE OSMOTIC PROPORTIONS OF THE NITROGEN BEARING SALTS OF THE CULTURE SOLUTIONS AND THE SOLUBLE ORGANIC AND THE AMMONIA NITROGEN IN THE PLANTS

two nitrogen fractions and the nitrate nitrogen of the solutions: high yields of these two fractions in the plants correspond to low proportions of nitrate nitrogen in the culture solutions, and low yields correspond to high proportions.

Insoluble organic nitrogen. This fraction represents the nitrogen found in the protoplasm, or the higher products of nitrogen synthesis. The numerical

TABLE 5

Insoluble organic nitrogen plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBERS	OSMOTIC PROPORTIONS				INSOLUBLE ORGANIC NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C
						per cent	*per cent*	*per cent*
1	12	3	3	1	3	3.17	2.77	1.63
	14	3	3	3	1	3 69	3.12	1.34
2	2	5	1	1	3	3.83	2.60	2.21
	5	5	1	3	1	4.14	3.39	2.28
3	18	1	5	1	3	3.14	3 04	1.70
	19	1	5	3	1	Lost	2.88	1.68
4	3	3	1	1	5	3.39	2.53	2.19
	8	3	1	5	1	3.83	3.27	2.05
5	13	1	3	1	5	3.62	2.85	1.77
	16	1	3	5	1	3.85	2.98	2.32
6	4	1	1	1	7	3.69	3.50	2.14
	10	1	1	7	1	3.98	2.84	Lost
7	7	1	1	3	5	3.81	3.33	1.98
	9	1	1	5	3	3.73	2.75	2.26
8	20	1	7	1	1	2.53	Lost	1.65
	1	7	1	1	1	3.33	3.31	2.09
9	17	3	5	1	1	3.62	2.91	1.69
	11	5	3	1	1	3.23	3.48	1.64
10	16	1	3	3	3	3.60	3.21	2.13
	6	3	1	3	3	3.80	3.12	2.45

data representing this fraction are given in table 5. As might be expected, for plants of a given age, these vital portions of the living cells are less influenced by the type of solutions in which the plants are grown than are the intermediate or lower forms of nitrogen compounds. There appears to be no definite relation between the insoluble organic nitrogen fraction in the plants and the proportions of nitrogen bearing salts in the culture solutions, although in the

young plants of series A four of the five highest yields of this fraction were produced by plants grown in solutions containing relatively high proportions of ammonium nitrogen and low proportions of nitrate nitrogen.

Although the yields of insoluble organic nitrogen in these plants fluctuate less between cultures of a given age within a series than do any of the other nitrogen fractions here considered, they vary more than do the yields of any other fraction between plants of corresponding cultures at different ages. The nitrogen in this fraction of oat seedlings when 20 days old represented an average of 3.65 per cent of their total dry weight. When the plants were 40 days old, in the early heading stage, this form of nitrogen had decreased to an average of 3.07 per cent. Then, as the plants passed the heading stage and approached maturity, there came a rapid decline in the insoluble organic nitrogen, so that on the date of harvest of series C it had decreased to an average of 1.9 per cent. This decline may be accounted for by the fact that as the plants approach maturity an increasing number of cells lose their protoplasmic content and the insoluble organic nitrogen decreases correspondingly for a given dry weight of tissue.

Total organic nitrogen. The close relationship which exists between the ammonium nitrogen of the solutions and the total organic nitrogen of the plants is evident from the data of table 6. High yields of this fraction correspond with high proportions of nitrogen as ammonium in the culture solutions and, in general, low yields correspond to low proportions of ammonium in these solutions.

These data of table 6 indicate that nitrogen as ammonia is more closely associated with the synthesis of proteinaceous compounds in the plant than is nitrate nitrogen, although, as has been shown previously, the inorganic nitrogen of the plant is composed largely of nitrate nitrogen. To emphasize further these relationships, the values of the total organic and the total inorganic nitrogen fraction of the plants of series A, B, and C are diagrammatically represented in figure 4 together with the osmotic proportions of the nitrogen bearing salts in the culture solutions. At the left are given the percentage values of total inorganic nitrogen, and on the right those of the total organic fraction. This diagram is constructed in precisely the same manner as that of figure 3.

It will be observed from figure 4 that both the total organic and the total inorganic nitrogen fractions are higher in the younger than in the older plants. A comparison of the diagrams representing the inorganic nitrogen values in series A and B with the relative proportions of nitrogen as nitrate in the culture solutions, shows that almost without exception high and low values of total inorganic nitrogen in the plants correspond, respectively, with high and low relative proportions of nitrate nitrogen in the culture solutions. The opposite relation is shown between total inorganic nitrogen in the plants and nitrogen as ammonium in the culture solutions. It is clear, then, that the nitrate nitrogen in the culture solutions, and not the nitrogen as ammonium, deter-

mines to a very large extent the total inorganic nitrogen of the plants. This follows from the fact that at any time nitrogen as ammonium comprises a very small proportion of the total inorganic nitrogen of the plants. On the other hand, by comparing the total organic nitrogen in the plants of series A and B with the two forms of nitrogen in the solutions, it will be observed that with very few exceptions, high and low total organic nitrogen in the plants corre-

TABLE 6

Total organic nitrogen in plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBERS	OSMOTIC PROPORTIONS				TOTAL ORGANIC NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C
						per cent	*per cent*	*per cent*
1	12	3	3	1	3	4.20	3.20	2.71
	14	3	3	3	1	4.48	3.22	2.64
2	2	5	1	1	3	4 53	3.24	2.92
	5	5	1	3	1	4.63	3.81	3.34
3	18	1	5	1	3	4.12	5.55	2.41
	19	1	5	3	1	Lost	3.63	2.61
4	3	3	1	1	5	4.27	3.13	2.96
	8	3	1	5	1	5.06	4.23	3.32
5	13	1	3	1	5	4.65	3.23	2.46
	16	1	3	5	1	5.00	3.58	4.29
6	4	1	1	1	7	4.70	3.56	3.03
	10	1	1	7	1	5.25	4.22	Killed
7	7	1	1	3	5	4.91	4.06	3.03
	9	1	1	5	3	4.93	3.86	3.85
8	20	1	7	1	1	4.43	3.16	2.22
	1	7	1	1	1	4.15	3.32	2.69
9	17	3	5	1	1	4 63	3.32	2.36
	11	5	3	1	1	4.19	3.66	2.21
10	15	1	3	3	3	4.81	3.86	3.22
	6	3	1	3	3	4.72	3.58	3.24

spond, respectively, with high and low relative proportions of nitrogen as ammonium in the culture solutions, whereas nitrate nitrogen in the solutions appears to have less influence upon the total organic nitrogen of the plants.

In general, the relations here pointed out for the plants of series A and B hold also for those of series C, but in the older plants these relations are not

quite so exact as they are in younger plants. This is to be expected, since, as already shown, the protoplasmic content of the older plants is low, as is their nitrate nitrogen content, whereas nitrogen as ammonia accumulates in their tissues—sometimes to the extent of actual toxicity—and thus it comprises a greater percentage of the inorganic nitrogen of the plants of series C than of the younger plants in series A and B.

TABLE 7

Total nitrogen in plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBER	OSMOTIC PROPORTIONS				TOTAL NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C
						per cent	*per cent*	*per cent*
1	12	3	3	1	3	5.71	4.58	3.70
	14	3	3	3	1	6.29	4.27	4.09
2	2	5	1	1	3	6.47	4.78	4.19
	5	5	1	3	1	6.43	4.91	4.47
3	18	1	5	1	3	6.12	5.39	3.81
	19	1	5	3	1	6.61	5.06	4.09
4	3	3	1	1	5	6.65	4.91	4.27
	8	3	1	5	1	6.80	5.45	4.91
5	13	1	3	1	5	6.63	4.62	3.87
	16	1	3	5	1	6.88	4.81	5.52
6	4	1	1	1	7	6.93	5.54	4.46
	10	1	1	7	1	7.00	5.45	Killed
7	7	1	1	3	5	7.08	5.80	4.84
	9	1	1	5	3	6.87	5.34	5.87
8	20	1	7	1	1	6.18	4.45	3.32
	1	7	1	1	1	5.79	4.46	3.84
9	17	3	5	1	1	6.47	4.85	3.39
	11	5	3	1	1	6.99	4.79	3.39
10	15	1	3	3	3	6.94	5.34	4.49
	6	3	1	3	3	6.97	4.96	4.77

Total nitrogen. The total nitrogen includes both the organic and the inorganic nitrogen fractions of the plants. The data relating to total nitrogen are presented in table 7. As previously shown, when the inorganic nitrogen of the plants has relatively high values the organic nitrogen is relatively low, and thus the total nitrogen of the plants within a given series fluctuates less than does any one of the fractions included in the total nitrogen. Since the

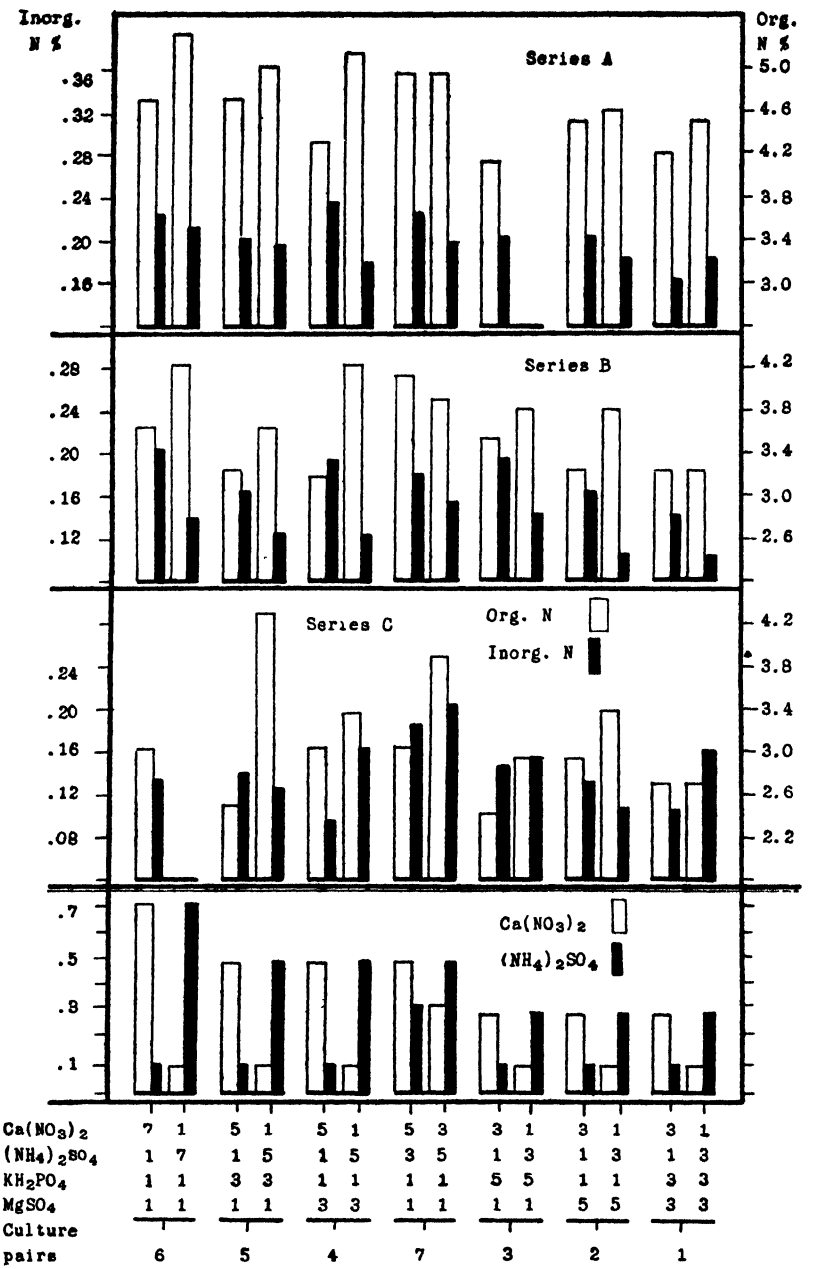

FIG. 4. DIAGRAM SHOWING THE RELATION BETWEEN THE OSMOTIC PROPORTIONS OF THE NITROGEN BEARING SALTS OF THE CULTURE SOLUTIONS AND THE TOTAL INORGANIC AND THE TOTAL ORGANIC NITROGEN IN THE PLANTS

larger part of the total nitrogen of the plants is in the organic form, and since organic nitrogen is closely correlated with nitrogen as ammonium in the culture solutions, it follows that the total nitrogen of the plants is influenced considerably more by the ammonium nitrogen than by the nitrate nitrogen of the solutions, although the latter exerts considerable influence in determining the total nitrogen of the plants, since the inorganic nitrogen fraction of the plants is largely determined by the nitrate nitrogen in the culture solution in which the plants were grown.

From a physiological point of view, the total nitrogen of the plant carries little significance, since it gives no clue to either the type or the quantity of nitrogen in any particular nitrogen fraction of the plant, and it is quite clear that it can not serve as an adequate criterion of any organic constituent of the plant when, as frequently found, more than one-third of the total nitrogen present in the plants may be inorganic in nature, largely in the form of nitrate nitrogen.

From the foregoing considerations, it is apparent that ammonium nitrogen is more closely associated with the synthesis of the organic nitrogenous compounds of the plant than is nitrate nitrogen. In the early stages of growth, when protein synthesis per gram of tissue is most rapid, the ammonia nitrogen is undoubtedly readily converted into the organic forms of nitrogen and very little of it accumulates in the tissue of the younger plants. Thus the plants in the solutions with high relative proportions of nitrogen as ammonium and low relative proportions of nitrate nitrogen made just as good growth as, and had a higher protein content and a lower content of inorganic nitrogen during the early phases of their development than, did the plants in solutions with low relative proportions of nitrogen as ammonium and high relative proportions of nitrogen as nitrate. In the later stages of growth when the plants are approaching maturity, the synthesis of nitrogenous compounds per gram of tissue is greatly reduced as compared with this process in younger plants. Thus the conversion of ammonia nitrogen to the organic forms is greatly retarded. In the older plants, grown in solutions with high relative proportions of nitrogen as ammonium and low relative proportions of nitrogen as nitrates, absorption of ammonium nitrogen apparently outstripped assimilation, with the result that ammonia nitrogen accumulates in the plant tissues in sufficient concentrations to retard growth materially, and sometimes to produce severe pathological conditions, whereas the same solutions often produce much better plants during the early stages of development than do solutions with high relative proportions of nitrogen as nitrate and low relative proportions of ammonium nitrogen.

SUMMARY

From the investigation reported in the foregoing pages, on the quantitative relationships between the inorganic nitrogen constituents of culture solutions of known composition and the various nitrogen fractions of oat plants produced by these solutions, the following relations may be briefly summarized:

In the younger stages of development, the oat plants grew equally well in the high ammonia and in the high nitrate solutions, but in the later stages of their development the growth of the plants in solutions high in ammonium was much less than that of plants in the high nitrate solutions.

Correlated with low dry weight yields of the older plants in the high ammonium solutions was a relatively high yield of nitrogen as ammonia in the plants, and this was the only nitrogen fraction considered which showed higher yields in the older than in the younger plants.

The nitrogen as ammonia in the plants was high or low, respectively, with high or low proportions of nitrogen as ammonium in the culture solutions in which the plants were grown.

The nitrate nitrogen in the plant was high or low, respectively, with a high or low amount of nitrate nitrogen in the culture solutions in which the plants were grown.

The inorganic nitrogen of these plants was largely in the nitrate form. It often represented more than 2 per cent of the dry weight of the plants and sometimes comprised more than one-third of the total nitrogen.

Plants high in soluble organic nitrogen had high ammonia content and were produced by solutions with high relative proportions of nitrogen as ammonium and low proportions of nitrate nitrogen.

For plants of a given age, the insoluble organic nitrogen fraction was more constant and less influenced by the chemical composition of the culture solutions in which the plants were grown than were any of the other nitrogen fractions; but since protein synthesis per gram of tissue was greatly reduced as the plants approached maturity, the insoluble organic nitrogen was more influenced by the age of the plant than was any other fraction.

Solutions with high relative proportions of nitrogen as ammonium produced plants which were high in total organic nitrogen and low in inorganic nitrogen, whereas solutions with high relative proportions of nitrate nitrogen produced plants high in inorganic and low in organic forms of nitrogen.

The total nitrogen of the plants varied with the total nitrogen of the solutions in which the plants were grown, but not in direct proportion to the amounts appearing in the culture solutions.

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STUDIES ON NITROGEN ABSORPTION FROM CULTURE SOLUTIONS: I. OATS[1]

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The investigations described in the following pages deal with a study of the absorption of nitrogen in the forms NH_4 and NO_3 by roots of plants grown in solution cultures.

The status of this problem of the utilization of ammonium and nitrate nitrogen by agricultural plants is still in a very unsatisfactory condition. Experimental evidence favors the idea that not only are ammonium salts as well as nitrates good sources of nitrogen for higher plants, but also that both forms are required under certain conditions by some plants and that plants differ in their ability to utilize ammonium as a source of nitrogen. Some plants appear to require ammonium during one stage of development and nitrate during another.

A review of the literature on this subject shows that the substitution of ammonium nitrogen, either partially or wholly, for nitrate nitrogen is a procedure which is not of recent origin. Hutchinson and Miller (7, 8) summarize the more definite contributions to the literature on this subject, as do Jones and Shive (10).

More recently Prince, Jones, and Shive (13) have shown that when ammonium sulfate is superimposed upon the Shive three-salt solution, or when this salt is substituted for the potassium nitrate of the Tottingham solution in equivalent osmotic concentrations, thus enabling the plants to obtain their nitrogen in the two forms, NH_4 and NO_3, the H-ion concentrations of the solutions in which the roots of the absorbing plants are immersed increase during the early stages of the growth of the plants, but the rate of increase is not nearly so marked as it is when NH_4 alone furnishes the source of nitrogen. However, when the plants reach a certain stage in their development, the direction of the reaction change is reversed, thus indicating a reversal in the NH_4 and NO_3 absorption rates. This occurs when the plants are 4 or 5 weeks old.

Jones and Skinner (9) in a recent investigation found that the relative absorption rate of ammonium nitrogen per unit area of absorbing surface decreases with the age of the plant, although it increases with respect to nitrate nitrogen absorption. They also observed that the rate of absorption of ammonium nitrogen does not materially increase with an increase in concentration of the ammonium salt in the solution, whereas, on the other hand, the rate of nitrate nitrogen absorption does increase markedly as the nitrate content of the medium increases.

The following investigations were undertaken for the purpose of studying further the relative rates of the absorption of nitrogen in these two forms,

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NH_4 and NO_3, by the roots of plants throughout the entire life cycle, when both forms are present in the culture solution and available to the plants. The studies were carried out with two species: oats (*Avena sativa*) and buckwheat (*Fagopyrum esculentum*). The results obtained with oats alone will be presented here; those obtained with buckwheat will appear in a later publication.

EXPERIMENTAL METHODS

Method of growing plants

A pure line of oat seeds of the Gopher variety were germinated on a net as described by Shive (14). Seedlings, carefully selected for uniformity of size and vigor, were transferred when about 5 cc. tall to a culture solution, after having been mounted in a double piece paraffined cork stopper devised by Tottingham (16). The stoppers were of the proper size to fit 2-quart fruit jars of colorless glass, which were used for culture vessels. Three seedlings were included in each culture. The cultures were grown in the green-house with full exposure to sunlight, the temperature ranging between 18°C. and 23°C.

The culture media for all the work were the modified Tottingham solution $T_1R_3C_5$ (10) containing $(NH_4)_2SO_4$, $Ca(NO_3)_2$, KH_2PO_4, and $MgSO_4$ in the following volume molecular proportions: 0.0042, 0.0075, 0.0021, and 0.0024, respectively; and solution $T_1R_3C_3$ with the volume molecular proportions of 0.0042, 0.0043, 0.0021, and 0.0071, respectively. Baker's analyzed salts were used in the preparation of all the solutions. The solutions were renewed continuously by the addition of 1 liter of fresh solution every 24 hours according to the "drip and drain" method at constant rates described by Shive and Stahl (15). This system of solution renewal also aerates the solution. Iron was supplied daily to all the cultures in the form of freshly prepared aqueous solution of ferrous sulfate, according to the need of the plant determined by its appearance. Boron was added in the form of boric acid in concentration of 1 p.p.m. In all cases, the solution used and the methods employed in the cultural procedure permitted an excellent development of the plants. The cultures were prepared and conducted as described in the foregoing through a time period of 12 days before the first absorption test was made.

Method of transferring cultures for the absorption tests

When the plants were 18 days old from the time of germination and 12 days in the culture jars, 24 cultures were selected in which the plants were about equal with respect to size and vigor in so far as this could be judged from careful observation. Two groups of 12 cultures each were selected. The three plants in each culture, together with the paraffined cork stoppers in which they were mounted, were taken from the 2-quart culture jars and transferred to pint jars containing exactly 500 cc. of the same solution as that in which

the plants were grown. By the continuous flow method of Shive and Stahl (15) 1 liter of new solution was then passed through each culture jar containing the measured 500 cc. of solution, in which the roots of the plant were immersed, during the absorption interval. The continuous flow method was necessary for reasons which will be made clear in a following section. Before the plants were transferred to the measured solutions, the roots were thoroughly rinsed with distilled water. This transfer of all the plants in both groups of a series was made at the time when the experiment began, always at 8:00 a.m. One culture was then removed from each group at the end of 4 hours, another at the end of 8 hours, and so on at 4-hour intervals up to 48 hours.

At the end of each absorption interval the plants were harvested and their green and dry weights obtained in the usual way. A new series of cultures was then started as before and the plants grown as before until they were 12 days older than those of the series which immediately preceded. This procedure was continued until the last series to be investigated was at or near the stage of maturity, when the absorption tests were made.

Methods of analyses

Immediately after the removal of the cultures from the series at the end of the various experimental periods, the solutions in which the plant roots had been immersed for definite intervals, together with two solution checks and a distilled water check, were analyzed for ammonium nitrogen, nitrate nitrogen, and total nitrogen. A 100-cc. aliquot was used for each analysis. The methods used were those which, after many preliminary tests, were found best adapted to determine small amounts of inorganic nitrogen, and which gave very small analytical error. Several methods were tried, but the experimental error in some of these was relatively high and could not compare with the following methods for accuracy:

Ammonium nitrogen determination. The Official Kjeldahl-Gunning-Arnold Method. (Association of Official Agricultural Chemists) (1) was employed with modifications in apparatus and quantities of distillate, acid and water used, as recommended by Paul and Berry (12).

Nitrate nitrogen determination. The Devarda Method [Association of Official Agricultural Chemists (2)], with modifications by Paul and Berry (12) as suggested in the foregoing.

The ammonium nitrogen was determined on a 100-cc. aliquot, cooled, and the reductor (Devarda's Alloy Metal containing Cu, 50 per cent; Zn, 5 per cent; and Al, 45 per cent, was added and the nitrate nitrogen determined following the official method as stated in the foregoing.

Total nitrogen determination. The total nitrogen was determined by the official method of the Association of Official Agricultural Chemists (3) and checked by adding together the ammonium and nitrate nitrogen values. All analytical values were checked very carefully with the theoretical values,

whenever this was possible, and if these did not correspond very closely the values were discarded. If the analytical values for the duplicate cultures did not check very closely with each other, these values were discarded. In general the values for duplicate cultures did check very closely, showing that differences in the rates of absorption between the cultures was not a disturbing factor in these experiments.

The acid and alkali used in titration were standardized against recrystallized Merck's Blue Label calcium carbonate. Twentieth normal acid and alkali were used in all titrations and the normality factors determined frequently to avoid possible error from this source. All titrations were carried out with a Schellbach burette and to complete disappearance of any red color. With twentieth normal alkali and acid, methyl red was found to be much more sensitive than cochineal or methyl orange as indicators.

Every determination was run in duplicate and repeated if checks were not obtained. The distilled water checks showed no nitrogen present in either the ammonium or the nitrate forms.

The periods at the different stages of development of the plants during which these experiments were carried out, will here be designated as *experimental periods*, and the time periods of immersion of the roots in the test solutions will be designated as *test* or *absorption intervals*.

Since duplicate cultures were employed during each absorption interval and since all analyses were carried out in duplicate, all the values represented in the tables are averages of at least four determinations involving two cultures and six plants. Since 24 cultures constituted a series for every experimental phase, all the final average values presented in the tables and those plotted and represented in graphs are averages of 48 determinations each involving 24 cultures and 72 plants.

All results were calculated (*a*) on the basis of actual nitrogen absorbed in the form in question, in milligrams per culture of three plants per hour, which will here be designated *quantity absorption*, and (*b*) on the basis of milligrams per gram of dry plant material per hour, which will be designated *rate absorption*. The data obtained by these two methods of calculation will be presented and considered separately.

EXPERIMENTAL RESULTS

Preliminary experiments

During some preliminary experiments, plants were transferred from the 2-quart culture jars in which they were grown with a continuously renewed solution, to the pint jars containing 500 cc. of solution, where they remained for the time of the absorption interval without continuous flow of solution through the culture jar. At the end of the test interval the plants were returned to the original 2-quart culture jars after the roots had been rinsed free from adhering solution with distilled water from a wash bottle, care being

taken to retain all the rinse water with the solution to be analyzed. Here they were grown for another 12 days in a continuously renewed solution, when a similar transfer and test was made. This procedure was continued at 12-day intervals throughout the course of these preliminary experiments, extending over a growth period of 78 days when the plants had just passed the blossoming stage. The modified Tottingham solution $T_1R_3C_5$ (10) was used throughout. Because of the water loss due to transpiration, distilled water was added to the culture jars at frequent intervals to maintain as nearly as possible the original volume of the test solution.

All results were here calculated only on the basis of nitrogen absorbed (removed from a fixed quantity of solution) in the form in question, in milligrams per culture per hour (quantity absorption). Since the plants were not harvested at the end of each test interval, dry weights could not be obtained upon which to calculate absorption rates on the basis of nitrogen absorbed per gram of dry plant material per hour.

Some of the data dealing with these preliminary experiments are here presented briefly for the sole purpose of bringing out and emphasizing several important points, the principal one of which deals with the influence of continuous renewal of test solutions, or the lack of it, upon absorption rates during the test intervals. These data are presented in table 1. As may be observed, each horizontal section of the table presents the data obtained during an experimental period including 12 absorption intervals ranging in time from 4 hours as the minimum to 48 hours as the maximum time during which the roots of the plants were exposed to the test solution. Both absorption tests and analytical determinations were carried out in duplicate; hence each value in the table except those in the last line of each horizontal section represents the average of four determinations. Each of the values in the last line of each horizontal section, marked *average*, represents the final average of 48 determinations involving 24 cultures and 72 plants.

The final average values for all the experimental growth stages as given in table 1, were plotted to form the graphs of figure 1. It will be observed that the graph representing the average hourly quantity intake of nitrogen as NH_4 shows a gradual decrease in values for the experimental stages until the plants had reached the age of 66 days, at which point the graph takes an abrupt rise. This indicates that up to this point the average hourly intake of nitrogen as NH_4 gradually decreases with the age of the plant. The abrupt break in the curve following the 66-day experimental stage immediately followed abundant tillering of the plants and the formation of much new and vigorous young tissue. The abrupt rise in this graph is undoubtedly the result of the formation of this young plant tissue with consequent increased nitrogen intake as NH_4 per culture per hour, since it is evident that under the conditions of these tests young growth absorbs nitrogen as NH_4 at a much higher rate than does the older growth. Hence, tillering, which represents young growth, abruptly reverses the direction of the graph of NH_4 absorption.

TABLE 1

Quantity absorption of nitrogen as NH_4 and NO_3 and total nitrogen per culture by oat plants during absorption intervals ranging in time from 4 hours to 48 hours, at different stages of development in the life cycle

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		NH_4, per culture		NO_3, per culture		Total N, per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
18	4	2.900	0.725	−0.444	−0.111	2.456	0.614
	8	4.188	0.524	−0.222	−0.028	3.966	0.496
	12	4.461	0.372	−0.111	−0.009	4.350	0.363
	16	4.961	0.210	−0.111	−0.007	4.850	0.203
	20	5.353	0.268	0.233	0.012	5.586	0.280
	24	5.688	0.237	0.667	0.028	6.357	0.265
	28	5.911	0.211	2.450	0.088	8.361	0.299
	32	6.002	0.188	2.004	0.063	8.006	0.251
	36	5.911	0.164	2.227	0.062	8.138	0.226
	40	5.799	0.145	3.006	0.075	8.805	0.220
	44	5 688	0.129	3.340	0.076	9.028	0.205
	48	5.799	0.121	4.230	0.088	10.029	0.209
Average			0.275		0.028		0.303
30	4	2.656	0.664	−0.273	−0.068	2.383	0.596
	8	3.149	0.394	−0.796	−0.099	2.353	0.295
	12	3.237	0.270	−0.441	−0.037	2.796	0.233
	16	3.415	0.213	0.276	0.017	3.691	0.230
	20	3.680	0.184	0.826	0.041	4.506	0.225
	24	4.034	0.168	1.419	0.059	5.453	0.227
	28	4.389	0.157	1.950	0.070	6.339	0.227
	32	4.831	0.151	1.773	0.055	6.604	0.206
	36	4.743	0.132	1.535	0.043	6.278	0.175
	40	4.831	0.121	2.127	0.053	6.958	0.174
	44	5.160	0.117	3.103	0.071	8.263	0.188
	48	4.983	0.104	3.634	0.076	8.617	0.180
Average			0.223		0.023		0.246
42	4	2.932	0.733	0.178	0.045	3.110	0.778
	8	3.502	0.438	−0.178	0.022	3.324	0.416
	12	4.114	0.343	−0.711	0.059	3.403	0.284
	16	4.114	0.257	−0.888	0.056	3.226	0.201
	20	5.446	0.272	−0.617	0.031	4.829	0.241
	24	5.859	0.244	0.000	0.000	5.859	0.244
	28	6.652	0.238	2.689	0.096	9.341	0.334
	32	5.509	0.172	3.376	0.106	8.885	0.278
	36	5.261	0.142	7.122	0.198	12.383	0.344
	40	5.261	0.132	11.103	0.278	16.364	0.410
	44	4.639	0.105	12.436	0.283	17.075	0.388
	48	4.639	0.097	12.791	0.267	17.430	0.364
Average			0.265		0.092		0.357

TABLE 1—*Concluded*

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		NH_4, per culture		NO_3, per culture		Total N, per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
54	4	1.518	0.380	−0.076	−0.019	1.442	0.361
	8	2.592	0.324	−0.132	−0.017	2.460	0.307
	12	3.037	0.253	2.978	0.248	6.015	0.501
	16	3.704	0.232	5.385	0.337	9.089	0.569
	20	3.333	0.107	5.755	0.288	9.088	0.395
	24	2.963	0.123	5.940	0.248	8.903	0.371
	28	2.592	0.093	6.607	0.236	9.199	0.329
	32	2.407	0.075	7.792	0.244	10.199	0.319
	36	2.592	0.072	10.015	0.278	12 607	0.350
	40	2.222	0.056	10.383	0.259	12.605	0.315
	44	2.073	0.047	11.309	0 257	13.382	0.304
	48	2.222	0.046	11.766	0.245	13.988	0.291
Average			0.151		0.217		0.368
66	4	0.504	0.126	5.023	1.256	5.527	1.382
	8	0.511	0.064	8.513	1.064	9.024	1.128
	12	0.507	0.042	10.652	0.888	11.159	0.930
	16	0.536	0.034	10.968	0.686	11.504	0.720
	20	0.511	0.026	11.099	0.555	11.610	0.581
	24	0.741	0.031	11.344	0.473	12.085	0.504
	28	1.926	0.069	10.009	0.357	11.935	0.426
	32	2.362	0.074	10.297	0.322	12.659	0.396
	36	4.228	0.117	11.629	0.323	15.857	0.440
	40	4.611	0.115	12.258	0.306	16.869	0.421
	44	4.228	0.096	12.693	0.288	16.921	0.384
	48	3.768	0.079	13.422	0.280	17.190	0.359
Average			0.073		0.567		0.639
78	4	2.932	0.733	2.449	0.612	5.381	1.345
	8	3.501	0.438	3.330	0.416	6.831	0.854
	12	3.344	0.279	4.342	0.362	7.686	0.641
	16	4.402	0.275	5.097	0.319	90.499	0.594
	20	5.381	0.268	6.365	0.318	11.746	0.586
	24	5.859	0.244	8.078	0.337	13.937	0.581
	28	5.859	0.209	11.750	0 420	17.609	0.629
	32	5.509	0.172	13.953	0.436	19.462	0.608
	36	5.461	0.152	15.177	0.422	20.638	0.574
	40	5.461	0.137	15.666	0.392	21.127	0.529
	44	4.640	0.105	16.646	0.378	21.286	0.483
	48	4.639	0.097	17.625	0.367	22.264	0.464
Average			0.259		0.398		0.657

On the other hand, the graph representing the absorption of nitrogen as NO_3 begins with very low values and gradually rises with increase in the age of the plants, reaching a maximum at the 66-day experimental stage, when the graph takes an abrupt downward direction which corresponds to the pronounced upward direction taken by the graph representing nitrogen absorption as NH_4 and coincides with the development of numerous tillers and abundant young growth by the plants. Thus during the early stages of growth a low quantity intake of nitrogen as NO_3 is indicated for the oat plant and a relatively high intake per culture per hour during the later stages of growth.

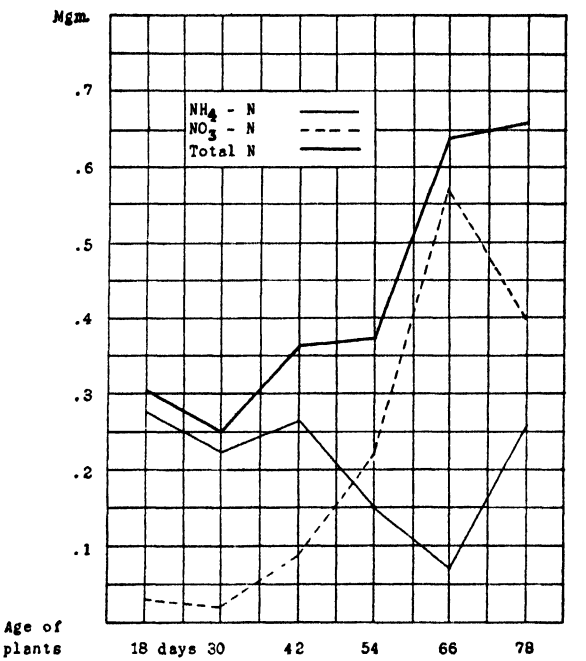

FIG. 1. GRAPHS REPRESENTING QUANTITY OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN ABSORBED, IN MILLIGRAMS PER CULTURE PER HOUR, AT DIFFERENT PERIODS IN THE LIFE CYCLE OF THE OAT PLANT

The graph representing total nitrogen intake per culture per hour takes a general upward slope from the point representing the first experimental growth stage to the point representing the last stage, indicating that the total nitrogen intake gradually increases with the development of the plants to the 78-day stage, when the plants had just passed through the flowering period. An accident necessitated the discontinuance of this series of cultures at the 78-day stage. However, as will be shown later, total nitrogen intake per culture per hour reaches its maximum for this plant at about this stage of development.

Irregularities in the graphs during the early growth phases may be traced to external conditions, particularly with respect to light intensity and temperature during the test periods, although no attempt was here made to measure the influence of these factors upon nitrogen absorption.

The important point to be emphasized here is the apparent high ability of vigorous young tissue, occurring either as the seedling phase of the life cycle or produced at a later stage of development, to absorb nitrogen as NH_4 almost to the exclusion of nitrogen as NO_3, when the two forms occur simultaneously in the culture medium. Equally interesting and important is the fact that the plants appear to lose this ability rapidly and apparently acquire increasing power to absorb nitrogen as NO_3 with increasing age of the tissues under the experimental conditions here defined. This latter point will be brought out more clearly and emphatically in a following section with a consideration of the actual absorption rates calculated on the basis of unit weight of dry plant tissue.

Influence of duration of absorption interval upon absorption rates

Absorption from a fixed quantity of solution without continuous solution renewal. The data of table 1 reveal the fact that during any one experimental period of 48 hours in the life cycle the quantity of nitrogen absorbed in milligrams per culture per hour decreases very markedly as the time of the absorption interval increases, except when the absorption values are very low. This occurs when absorption takes place from a fixed quantity of solution, limited in volume, without continuous renewal of solution. To illustrate these relations conveniently and clearly, the quantity absorption values per culture per hour as given in table 1, for the experimental periods at 18 days and at 66 days have been plotted to form the two sets of graphs given in figure 2. The data for only these two experimental periods are here presented in graphic form since they are representative of the data obtained for all the experimental periods during the life cycle of the plants, and the data for the remaining periods may very well be omitted from the discussion without detracting from the general significance of the results indicated by the graphs of figure 2.

As previously described, the cultures, each consisting of three plants, were placed in half-liter jars each containing 500 cc. of solution where they remained during the respective absorption intervals without solution renewal. At the end of the intervals the plants were removed, the solutions analyzed, and the absorption data obtained.

From the graphs corresponding to the experimental period at 18 days it will be observed that the quantities of nitrogen absorbed as NH_4 per culture per hour gradually decrease as the length of the absorption intervals increases. On the other hand, the corresponding NO_3 absorption values show only slight variation as the length of the absorption interval increases. The NO_3 values throughout this experimental period were very low, whereas the NH_4 values were relatively high, as indicated by the graphs.

The graphs representing the corresponding data for the experimental period when the plants were 66 days old appear in a position which is just the reverse of that of the preceding graphs for the period at 18 days. Here the graphs of

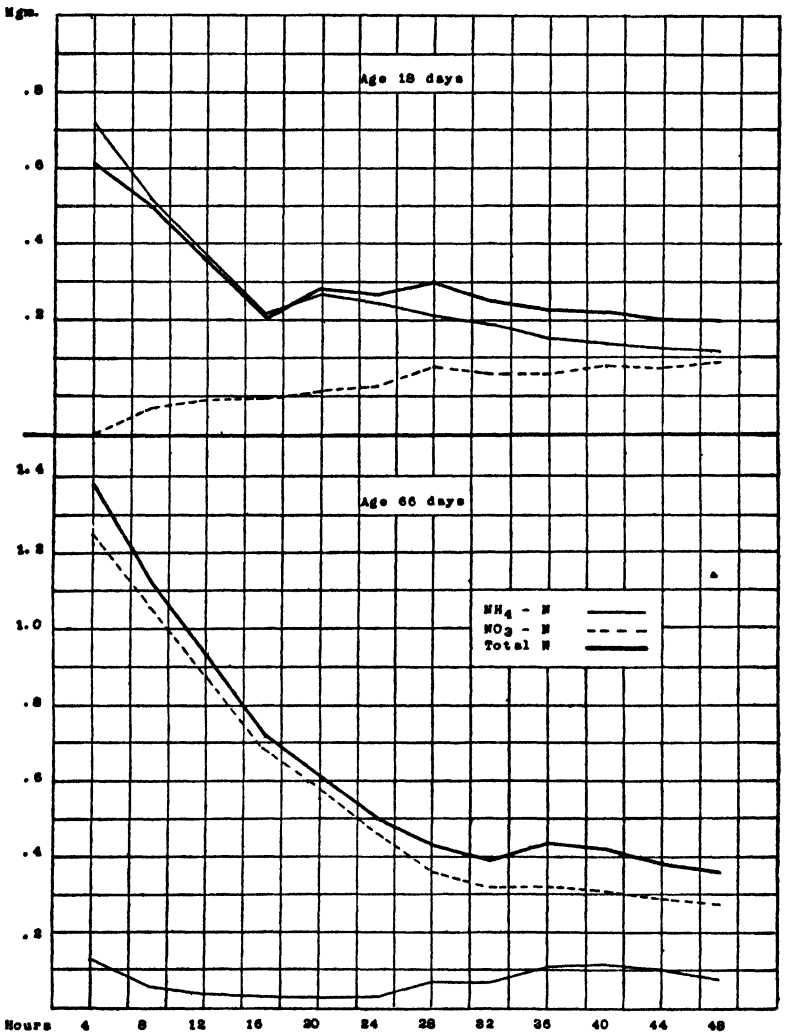

FIG. 2. GRAPHS SHOWING THE INFLUENCE OF DURATION OF ABSORPTION INTERVALS ON NITROGEN ABSORPTION RATES WITHOUT CONTINUOUS RENEWAL OF CULTURE SOLUTIONS

the absorption quantities of NO_3 in milligrams per culture per hour show values which are much higher than those of the corresponding NH_4 values throughout the entire experimental period of 48 hours. The average hourly NO_3 values now gradually decrease as the time of the absorption intervals in-

creases. During this experimental period the NH_4 values are all noticeably low and show only slight differences.

From a consideration of the graphs of figure 2, it appears that whenever the two forms of nitrogen here employed occur simultaneously in the culture solution and one form is absorbed by the plant roots from a fixed quantity of solution, without continuous renewal, in relatively low quantities and the other in relatively high quantities, the average hourly absorption of the latter decreases markedly with increase in the length of the time interval of absorption while that of the former is either not at all or only slightly influenced by variations in the length of the absorption interval. This is perhaps what might be expected on the assumption that change in concentration in the external medium through absorption of the particular constituent in question correspondingly changes the hourly absorption of this constituent during any given experimental period. But the matter appears to be not so simple as this. There can be no disputing the fact that quantity absorption per culture per hour is very materially influenced also from interval to interval by external variable factors such as light intensity in particular, temperature, and humidity, and this has been the main reason for adopting, in these investigations, a relatively large number of test intervals with a very wide range in time values (4 to 48 hours) during each experimental period throughout the life cycle of the plants. No attempt has here been made, of course, to evaluate these factors in their relation to nitrogen absorption.

As has already been pointed out, quantity absorption of nitrogen per calture per hour in the two forms here used, form identical solutions, changed markedly with the age of the plant, as is indicated by the complete reversal of the position of the graphs representing NH_4 absorption and NO_3 absorption when the plants were 18 days old and when they were 66 days old; but this is not a question of concentration. That the change in concentration of the nitrogen or other elements in the external medium due to absorption by the plant roots is the main factor which brings about this variation in the average hourly absorption with variable absorption intervals during an experimental period of 48 hours is clearly brought out by a similar consideration of corresponding data of absorption from fixed quantities of solution but continuously renewed during the absorption intervals.

Absorption from a fixed quantity of solution with continuous solution renewal. The data represented by the graphs of figure 3, correspond in every respect with those represented by the graphs of figure 2, except that the former were obtained from cultures in which the plant roots of each were immersed in a fixed quantity of solution which was continuously renewed during the absorption intervals, while the latter were obtained from cultures in which the roots of the plants were exposed to the same fixed quantity of solution but without continuous flow during the test intervals.

The absorption tests were here made by immersing the plant roots of each culture in 500 cc. of solution as before and then passing through the culture

jar, by the method of continuous flow at a constant rate described by Shive and Stahl (15), 1 liter of new solution during the absorption interval. The solution drained from the culture jar during the test interval was caught and

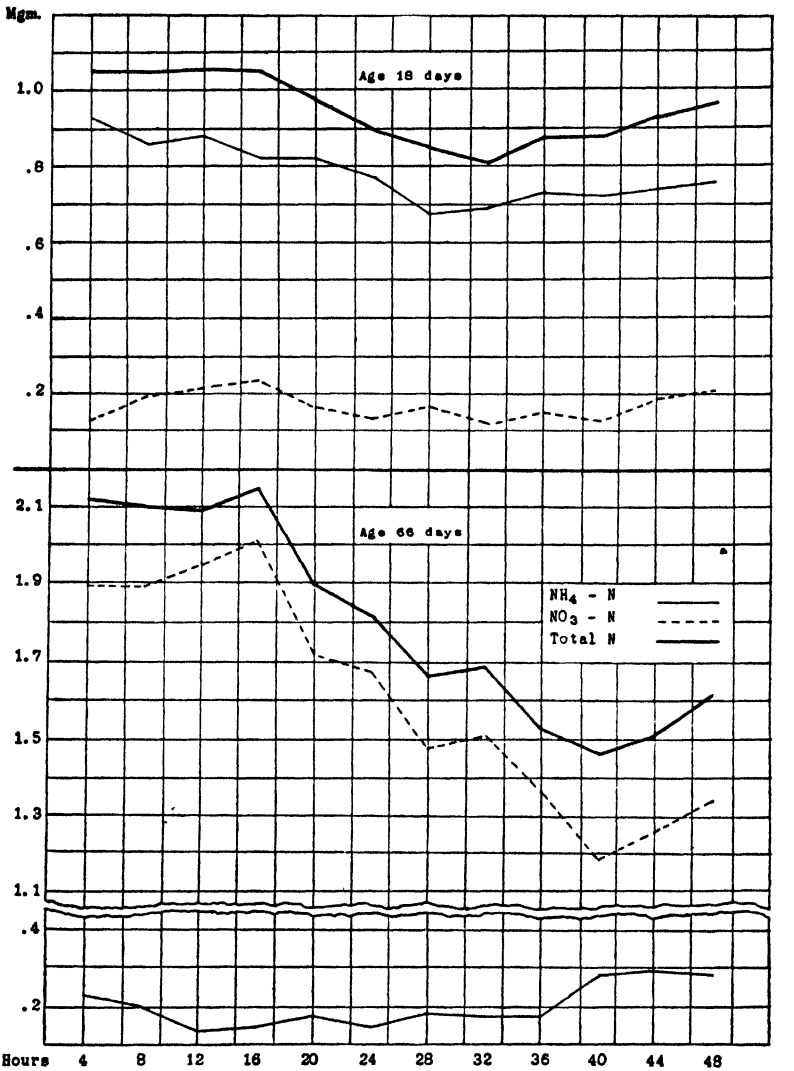

FIG. 3. GRAPHS SHOWING THE INFLUENCE OF DURATION OF ABSORPTION INTERVALS ON NITROGEN ABSORPTION RATES WITH CONTINUOUS RENEWAL OF CULTURE SOLUTIONS

added to the 500 cc. remaining in the culture jar at the end of the test. Analyses were then made on the whole 1,500 cc. of test solution. This method was used in all of the work here reported except that of the preliminary experiments already described.

The data, for the experimental period of 48 hours at 18 days and at 66 days, represented by the graphs of figure 3, were taken from table 2, and correspond to the data represented by the graphs of figure 2 taken from table 1.

From the graphs of figure 3, representing the data for the 18-day experimental period, it will be observed that with continuous flow there is no significant variation in the nitrogen absorption in milligrams per culture per hour with increase in length of time of the test intervals. This is indicated by the approximately horizontal position of the graphs.

The graphs representing the data for the 66-day period show a slight decrease in the absorption of total and NO_3 nitrogen in milligrams per culture per hour as the time duration of the test intervals increases, as is indicated by the downward pitch of the graphs following the 16-hour absorption interval. Assuming, of course, that decrease in the absorption values indicated by the graphs is the result of change in concentration due to absorption, this shows that continuous flow of solution, even at the rate here employed, does not sufficiently overcome concentration change during the longer test intervals to counteract completely its retarding influence upon absorption rates. Here, of course, total and NO_3 absorption values are considerably higher than are total and NH_4 absorption values during the 18-day period.

Here again, as previously, the relative positions of the graphs indicate that total nitrogen absorption is dominated by NH_4 absorption during the early stages of development—the 18-day period—, whereas during the later growth phases—the 66-day period—, NO_3 absorption dominates total nitrogen absorption.

A comparison of the graphs of figure 2 with the corresponding graphs of figure 3 brings out very clearly the importance of some system of continuous flow solution renewal during investigations of the nature here described, and the data represented by these two sets of graphs are here presented to emphasize this importance. There can be little doubt that continuous flow is very important for the growth of experimental plants in general where culture solutions or sand cultures supplied with culture solutions are involved.

Consideration of nitrogen excretion. In table 1 some negative values are indicated for quantity absorption of NO_3-nitrogen during several experimental periods in the life cycle. These negative values are usually shown for the short time test intervals only, and appear to indicate excretion of nitrogen from the plant roots into the test solution. The regular occurrence of this phenomenon throughout the preliminary investigations without continuous solution renewal during the test intervals with both oats and buckwheat, when a fixed quantity of test solution (500 cc.) was employed with each culture, is fairly good evidence that these negative values, although small, are not purely accidental or due to experimental error, but probably have some significance.

It has been suggested (4) that under such conditions it is probable that some of the external root cells, as they die, and are sloughed off, give up their

solutes to the surrounding solution. It has also been suggested (5, 6) that the ions given off by the plant roots may be the result of leaching from the dead cells or exchange of ions in the cell wall or protoplasm. Whether these suggestions could explain the presence of the excess nitrogen found in the test solutions after contact with the plant roots, is questionable. At present no explanation of the phenomenon here observed can be offered.

It is important to point out, however, that in the absorption series to be described in the following sections, in which the method of continuous solution renewal at constant rates (15) was employed during the absorption intervals while the plant roots were in contact with the test solutions negative absorption (excretion) of nitrogen did not occur. Thus the importance of negative values in this connection largely disappears.

The absorption series

Introductory. The plants of these series were tested at seven different stages of development throughout the life cycle. This required seven different series of cultures, since the plants were harvested and their dry weights obtained at the end of each experimental period. Each series consisted of 12 cultures of three plants each, and each series was conducted in duplicate. The plants of the first series were tested when the plants were 18 days old. Those of the second series were tested at the age of 30 days, and so on at 12-day intervals, throughout the life cycle. The tests of the last series were made when the plants were 102 days old, having produced mature seed.

As in the preliminary experiments, each test extended over a period of 48 hours, the absorption intervals during each test ranging in time from 4 hours, which was the minimum time of exposure, to 48 hours, which was the maximum absorption interval as previously described. The modified Tottingham solution $T_1R_3C_3$ (10), which contained approximately equal proportions of nitrogen as NH_4 and as NO_3, was used as the growth medium of the plants in each of the seven series and also as the test solution during the seven experimental periods in the life cycle. Throughout the test intervals the plant roots of each culture were immersed in a fixed quantity (500 cc.) of solution which was continuously renewed by passing through the culture jar at a constant rate of flow 1,000 cc. of new solution during the respective test interval. The total quantity, 1,500 cc. of solution, to which the roots of a culture were exposed during the test interval was used in making the chemical analyses.

The results of the chemical analyses were calculated, first, on the basis of absolute quantities of nitrogen absorbed in the different forms per culture per hour. Since the plant as a whole is a variable quantity, increasing in size and weight with age, results obtained by this method indicate only the course of quantity nitrogen absorption during the life cycle. The results were calculated, second, on the basis of nitrogen absorbed in the different forms per unit quantity of dry plant tissue per hour. This method of calculation deals with actual absorption rates and indicates the activity of the

TABLE 2

Quantity absorption of nitrogen as NH_4, NO_3, and total nitrogen per culture by oat plants during absorption intervals ranging in time from 4 hours to 48 hours, at different stages of development throughout the life cycle

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		Ammonia per culture		Nitrate per culture		Total nitrogen per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
18	4	3.681	0.928	0.532	0.133	4.214	1.054
	8	6.837	0.855	1.596	0.199	8 433	1.054
	12	10.518	0.877			10.518	0.877
	16	13.148	0.821	3.723	0.233	16.871	1.054
	20	16.304	0.815	3.191	0.160	19.469	0.975
	24	18.407	0.767	3.191	0.133	21.599	0.900
	28	18.933	0.676	4.786	0.171	23.720	0.848
	32	22.088	0.690	3.723	0.116	25.811	0.807
	36	26.295	0.730	5.319	0.148	31.614	0.878
	40	28.925	0.723	5.319	0.133	34.244	0.856
	44	32.660	0.741	8.510	0.193	41.117	0.934
	48	36.287	0.756	10.106	0.211	46.393	0.967
Average			0.780		0.152		0.934
30	4	3.156	0.789	1.596	0.399	4.752	1.188
	8	7.889	0.986	3.096	0.387	10.985	1.373
	12	8.415	0.701	4.256	0.355	12.671	1.056
	16	7.889	0.493	3.629	0.227	11.518	0.720
	20	11.570	0.579	4.787	0.239	16.357	0.818
	24	15.251	0.636	3.723	0.155	18.974	0.791
	28	12.621	0.451	5.319	0.190	17.940	0.641
	32	18.407	0.575	5.756	0.180	24.163	0.755
	36	16.304	0.453	6.383	0.177	22.687	0.630
	40	17.355	0.434	7.446	0.186	24.801	0.620
	44	19.458	0.442	6.833	0.155	26.291	0.598
	48	17.355	0.362	9.575	0.200	26.930	0.561
Average			0.575		0.238		0.813
54	4	4.659	1.165	4.437	1.109	9.096	2.274
	8	4.659	0.582	4.437	0.555	9.096	1.137
	12	7.764	0.647	10.871	0.906	18.635	1.553
	16	9.096	0.569	14.199	0.888	23.295	1.456
	20	8.652	0.433	18.636	0.932	27.288	1.364
	24	9.540	0.398	17.748	0.740	27.288	1.137
	28	8.874	0.317	19.079	0.681	27.953	0.998
	32	8.652	0.270	29.507	0.922	38.159	1.193
	36	8.430	0.234	29.951	0.832	38.381	1.066
	40	8.430	0.211	31.725	0.793	40.155	1.004
	44	8.208	0.187	33.572	0.763	41.780	0.950
	48	8.600	0.179	36.162	0.753	44.762	0.933
Average			0.432		0.824		1.255

TABLE 2—*Continued*

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		Ammonia per culture		Nitrate per culture		Total nitrogen per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
66	4	0.920	0.230	7.584	1.896	8.504	2.126
	8	1.608	0.201	15.167	1.896	16.775	2.097
	12	1.608	0.134	23.439	1.953	25.043	2.087
	16	2.298	0.144	32.172	2.011	34.470	2.154
	20	3.447	0.172	34.470	1.724	37.917	1.896
	24	3.447	0.144	39.986	1.666	43.433	1.810
	28	5.055	0.181	41.364	1.477	46.419	1.658
	32	5.745	0.180	48.258	1.508	54.003	1.688
	36	5.975	0.166	48.800	1.356	54.775	1.522
	40	11.490	0.287	47.407	1.185	58.897	1.473
	44	12.869	0.293	55.382	1.259	68.251	1.511
	48	13.329	0.278	64.344	1.341	77.673	1.618
Average			0.201		1.606		1.807
78	4	2.933	0.733	7.332	1.833	10.265	2.566
	8	2.933	0.366	9.971	1.246	12.904	1.612
	12	2.933	0.244	17.595	1.466	20.528	1.710
	16	2.933	0.183	17.595	1.100	20.528	1.283
	20	5.279	0.264	17.595	0.880	22.874	1.144
	24	5.573	0.232	25.062	1.044	30.635	1.276
	28	2.933	0.105	33.431	1.194	36.364	1.299
	32	2.933	0.092	40.032	1.251	42.965	1.343
	36	5.573	0.155	38.123	1.059	43.696	1.214
	40	8.798	0.183	52.785	1.320	61.583	1.503
	44	9.384	0.213	54.252	1.233	63.636	1.446
	48	11.144	0.232	61.583	1.283	72.727	1.515
Average			0.250		1.242		1.493
90	4	1.596	0.399	4.106	1.027	5.702	1.426
	8	3.069	0.384	7.038	0.880	10.107	1.263
	12	1.596	0.133	9.971	0.831	11.567	0.964
	16	2.346	0.147	11.730	0.733	14.076	0.880
	20	2.639	0.132	11.730	0.587	14.369	0.719
	24	2.346	0.198	21.114	0.880	23.460	0.978
	28	2.054	0.073	32.408	1.157	34.462	1.231
	32	1.467	0.046	39.590	1.237	41.057	1.283
	36			35.190	0.978	35.190	0.978
	40	2.625	0.066	45.161	1.129	47.786	1.195
	44	0.294	0.007	45.455	1.033	45.749	1.040
	48	2.640	0.055	52.785	1.100	55.425	1.155
Average			0.128		0.964		1.092

TABLE 2—*Concluded*

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		Ammonia per culture		Nitrate per culture		Total nitrogen per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	4	1.331	0.333	1.775	0.444	2.506	0.777
	8	1.553	0.194	4.881	0.610	6.434	0.804
	12	1.553	0.129	8.438	0.703	9.991	0.833
	16	1.331	0.083	11.537	0.721	12.868	0.804
	20	1.997	0.100	15.752	0.788	17.749	0.888
102	24	4.437	0.185	15.530	0.647	19.967	0.832
	28	4.437	0.159	17.526	0.626	21.963	0.784
	32	4.437	0.139	23.295	0.728	27.732	0.867
	36	3.993	0.111	23.960	0.666	27.953	0.777
	40	3.771	0.094	22.185	0.555	25.956	0.649
	44	2.219	0.050	22.851	0.519	25.070	0.570
	48	2.441	0.051	23.295	0.485	25.736	0.536
Average			0.136		0.624		0.760

plants with respect to nitrogen intake at the different stages of development in the life cycle. The results obtained by the two methods of calculation will be presented and considered separately.

Absorption of nitrogen per culture per hour (quantity absorption). The data dealing with the absolute quantities of nitrogen absorbed in the two forms, in milligrams per culture per hour, are presented in table 2. The data in this table are arranged in precisely the same manner as are those in table 1. As in the preceding table, each of the values except those marked "average," represents the average of four determinations. Each of the values marked "average" in the table represents the final average value of 48 determinations involving 24 cultures and 72 plants. These final average values only will be considered. They were plotted to form the graphs of figure 4.

It will be observed that the graph representing the average quantity absorption of nitrogen as NH_4 per culture per hour shows a gradual decrease in values for the experimental stages from the first stage at 18 days to the last at 102 days. It indicates during the early stages of growth a high quantity absorption of nitrogen as NH_4 per culture per hour which gradually decreases with the age of the plant. It is thus apparent that the maximum absorption of nitrogen as ammonia per culture per hour occurs at a very early stage of development and while the plants are still very young and small, in spite of the fact that the size, weight, and area of absorbing surface of the plant rapidly increase with age. During the later stages of growth the quantity intake of nitrogen as NH_4 becomes very low but at no time during the active life cycle does the plant entirely cease to absorb nitrogen as NH_4.

On the other hand, the graph representing the average quantity absorption of nitrogen as NO_3 per culture per hour begins with very low values and rises rapidly with increase in size, weight, and area of absorbing surface as the plants become older, reaching a maximum point at the 66-day stage. Following this, the graph takes a steep downward pitch, the average values decreasing with the age of the plant from this point. Thus during the early stages of growth a low average NO_3 absorption per culture per hour is indicated,

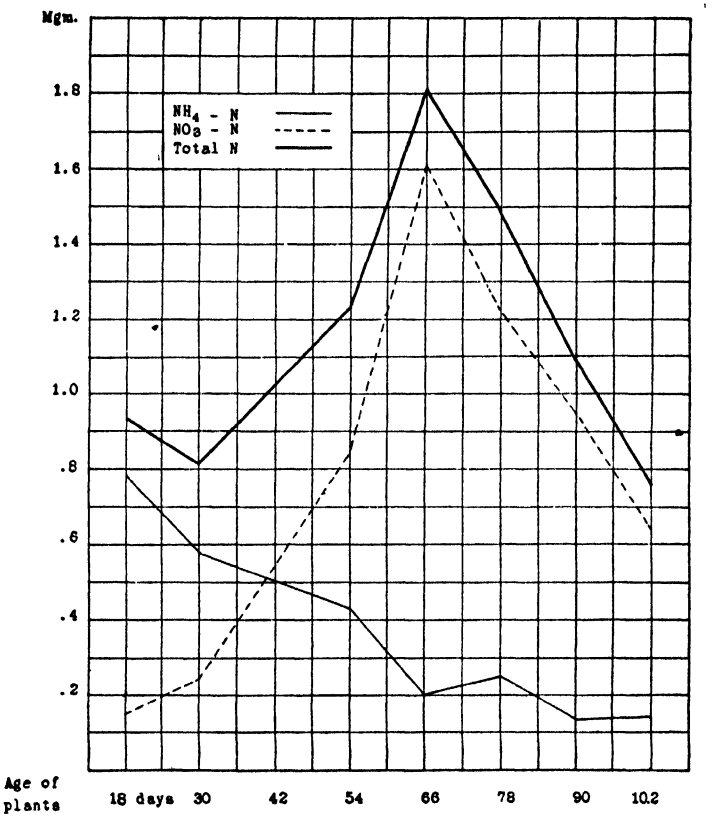

FIG. 4. GRAPHS REPRESENTING QUANTITY OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN ABSORBED, IN MILLIGRAMS PER CULTURE PER HOUR AT DIFFERENT PERIODS THROUGHOUT THE LIFE CYCLE OF THE OAT PLANT

with rapidly increasing values up to and including the reproductive phase at 66 days, after which the absorption values decrease rapidly with the age of the plants, as indicated by the steep downward pitch of the graph.

The graph representing average total nitrogen absorption per culture per hour in figure 4 first takes a downward slope between the 18-day and the 30-day period. The graph thus indicates a lower quantity absorption of total nitrogen when the plants were 30 days old than when they were only 18 days

old. This lower average intake at 30 days is directly correlated with a period of low light intensity, since the tests were made at this time during a period of cloudy weather and high humidity. This is an indication that external conditions, particularly conditions of light intensity, have a pronounced influence upon the absorption of nitrogen by the plants. The graph takes a steep upward slope from the point representing the 30-day period to that representing the 66-day period, the latter occurring during the time of flowering. At this point the maximum quantity absorption of total nitrogen per culture per hour is reached. This corresponds also to the point indicating the maximum absorption of nitrogen as nitrate. After the maximum point is reached, a steep downward slope is taken by the graph, indicating the rapid decrease in total nitrogen absorption per culture per hour during the late stages of growth and maturity. It is thus clear that the course of this graph representing the early stages of growth is determined mainly by quantity absorption of nitrogen as NH_4, whereas during the period of blossoming and later development the course of the graph is determined by quantity absorption of nitrogen as NO_3. The graphs of figure 4 very clearly bring out the fact that in a medium in which nitrogen is simultaneously present in the forms of ammonium and nitrate in approximately equal proportions, the plants while young satisfy the nitrogen requirement mainly through the absorption of ammonium, whereas during the later stages of development the plants obtain their nitrogen largely through the absorption of nitrate. However, at no time during the active growth cycle is the absorption of nitrogen either as ammonium or as nitrate completely at a standstill under the conditions of these experiments.

Absorption of nitrogen per unit of dry plant material per hour (rate absorption). The data dealing with the actual rates of absorption of nitrogen in the different forms calculated on the basis of intake per gram of dry plant material per hour are presented in table 3. As before, only the final average values as given in the table will here be considered. For the sake of convenience, the values representing nitrogen absorbed as NH_4, in milligrams per gram of dry plant material per hour will be designated the NH_4-rate, and the values representing nitrogen absorbed as NO_3 in milligrams per gram of dry plant material per hour will be designated the NO_3-rate. The final average rate values as given in table 3, were plotted to form the graphs of figure 5.

It will be observed that the course of the graph representing NH_4-rates of absorption per gram of dry plant tissue per hour shows a very steep downward slope at first and then a more gradual downward slope from the 30-day period to the 66-day period, and shows very low rates during the later phases of growth. Thus the graph indicates exceptionally high NH_4-rates during the early growth phases and very low NH_4-rates during the later phases of growth from the flowering period to maturity.

On the other hand, the graph representing the NO_3-rates, per gram of dry plant material per hour, shows very low rates at first which gradually rise

TABLE 3

Absorption of nitrogen as NH_4, NO_3, and total nitrogen per gram of dry plant tissue by oats during absorption intervals ranging in time from 4 hours to 48 hours at different stages of development throughout the life cycle

AGE OF PLANTS	ABSORPTION INTERVALS	DRY WEIGHT PER CULTURE	ACTUAL NITROGEN ABSORBED—MGM. PER GRAM DRY WEIGHT PER HOUR		
			NH_4–N	NO_3–N	Total N
days	*hours*	*gm.*			
18	4	0.587	1.567	0.226	1.794
	8	0.583	1.466	0.342	1.808
	12	0.599	1.464		1.464
	16	0.595	1.380	0.391	1.771
	20	0.586	1.391	0.272	1.663
	24	0.576	1.332	0.231	1.563
	28	0.565	1.197	0.303	1.500
	32	0.573	1.206	0.203	1.409
	36	0.584	1.251	0.253	1.504
	40	0.573	1.262	0.232	1.494
	44	0.578	1.283	0.335	1.618
	48	0.582	1.298	0.361	1.660
Average		0.582	1.341	0.262	1.604
30	4	1.224	0.645	0.326	0.971
	8	1.575	0.626	0.246	0.872
	12	1.414	0.496	0.251	0.747
	16	1.210	0.408	0.188	0.595
	20	1.405	0.412	0.170	0.582
	24	1.436	0.443	0.108	0.551
	28	1.201	0.475	0.158	0.534
	32	1.486	0.387	0.121	0.508
	36	1.497	0.303	1.118	0.421
	40	1.398	0.310	0.133	0.444
	44	1.475	0.300	0.105	0.405
	48	1.246	0.390	0.160	0.550
Average		1.381	0.416	0.174	0.590
54	4	2.432	0.479	0.456	0.935
	8	2.310	0.252	0.240	0.492
	12	2.294	0.282	0.395	0.677
	16	2.257	0.252	0.393	0.645
	20	2.453	0.176	0.380	0.556
	24	2.101	0.189	0.352	0.541
	28	2.014	0.157	0.338	0.496
	32	2.219	0.122	0.416	0.537
	36	2.240	0.105	0.371	0.476
	40	2.306	0.091	0.344	0.435
	44	2.315	0.081	0.330	0.410
	48	2.194	0.082	0.343	0.425
Average		2.261	0.189	0.363	0.552

TABLE 3—*Continued*

AGE OF PLANTS	ABSORPTION INTERVALS	DRY WEIGHT PER CULTURE	ACTUAL NITROGEN ABSORBED—MGM. PER GRAM DRY WEIGHT PER HOUR		
			NH_4–N	NO_3–N	Total N
days	*hours*	*gm.*			
	4	2.843	0.081	0.669	0.750
	8	2.792	0.072	0.679	0.751
	12	2.833	0.047	0.689	0.737
	16	2.714	0.053	0.741	0.794
	20	2.649	0.065	0.651	0.716
66	24	2.704	0.053	0.616	0.667
	28	2.640	0.068	0.560	0.628
	32	2.520	0.071	0.599	0.670
	36	2.510	0.066	0.540	0.606
	40	2.772	0.036	0.428	0.531
	44	2.790	0.048	0.451	0.556
	48	2.508	0.107	0.535	0.645
Average		2.690	0.075	0.596	0.671
	4	3.153	0.233	0.581	0.814
	8	3.082	0.119	0.404	0.523
	12	3.178	0.077	0.461	0.538
	16	3.052	0.060	0.360	0.421
	20	2.660	0 099	0.331	0.430
	24	3.004	0.077	0.348	0.425
78	28	3.024	0.035	0.395	0.430
	32	3.012	0.030	0.415	0.446
	36	2.940	0.053	0.360	0.413
	40	3.120	0.059	0.423	0.482
	44	3.080	0.069	0.400	0.470
	48	3.193	0.073	0.402	0.475
Average		3.042	0.082	0.407	0.489
	4	3.420	0.117	0.300	0.417
	8	3.396	0.113	0.259	0.372
	12	3.384	0.039	0.246	0.285
	16	3.322	0.044	0.221	0.265
	20	3.275	0.043	0.179	0.219
	24	3.320	0.030	0.264	0.294
90	28	3.298	0.023	0.251	0.374
	32	3.495	0.013	0.354	0.367
	36	3.341		0.292	0.292
	40	3.431	0.019	0.329	0.348
	44	3.358	0.002	0.308	0.310
	48	3.409	0.016	0.323	0.339
Average		3.371	0.038	0.285	0.323

TABLE 3—*Concluded*

AGE OF PLANTS	ABSORPTION INTERVALS	DRY WEIGHT PER CULTURE	ACTUAL NITROGEN ABSORBED—MGM. PER GRAM DRY WEIGHT PER HOUR		
			NH_4–N	NO_3–N	Total N
days	*hours*	*gm.*			
	4	4.103	0.081	0.108	0.189
	8	4.260	0.046	0.143	0.188
	12	4.295	0.030	0.133	0.163
	16	4.194	0.020	0.172	0.192
	20	4.302	0.023	0.183	0.206
102	24	4.148	0.045	0.156	0.201
	28	4.086	0.039	0.153	0.129
	32	4.242	0.033	0.172	0.204
	36	4.160	0.027	0.160	0.187
	40	4.156	0.023	0.133	0.156
	44	4.123	0.012	0.126	0.138
	48	4.034	0.013	0.120	0.133
Average		4.175	0.032	0.147	0.179

with increase in the age of the plant, reaching a maximum point at the 66-day experimental period, or the flowering stage of the plants. Thereafter the NO_3-rates decrease in value as the plants approach maturity. Thus during the early stages of growth, low NO_3-rates of absorption are indicated for the oat plant, relatively high rates during the reproductive phase, with declining rates from the blossoming stage to maturity.

The graph representing rates of total nitrogen absorption takes a very steep downward slope from the point representing the 18-day period to that representing the 30-day period, with a secondary maximum point at the 66-day period. The graph then shows continuously declining rates as the plants approach maturity. The secondary maximum point in the graph of total nitrogen absorption rates is the result of the high NO_3-rates at this point.

A consideration of these rate graphs makes it clear that the rates of nitrogen absorption by the young plants are completely dominated by the NH_4-rates, whereas from the blossoming period to maturity nitrogen absorption is dominated by the NO_3-rates, under the conditions of these experiments when the two forms of nitrogen are present in the external medium in approximately equal proportions. The graphs further show that the maximum NH_4-rate, which occurs in the very young plants, is more than double the maximum NO_3-rate, which occurs during the blossoming period. There are thus indicated two periods in the life cycle when the plants show higher activity with respect to nitrogen absorption than they do at other periods: the highest activity occurring at a very early stage, and the second period of high activity occurring during the blossoming stage.

A comparison of the graphs of figure 2 with those of figure 3 brings out the fact that the maximum total quantity of nitrogen absorbed per culture in unit

time occurs at the same point in the life cycle (blossoming period) as does the maximum NO_3-rate but this does not represent the maximum rate of total nitrogen absorption, which, as indicated by the rate graphs of figure 3, occurs at the earliest period in the life cycle here investigated and corresponds with the occurrence of the maximum NH_4-rate.

It is, of course, to be emphasized in this connection that the results here obtained apply only to the oat plant grown in a culture solution in which the nitrogen is simultaneously present in the two forms in approximately equal

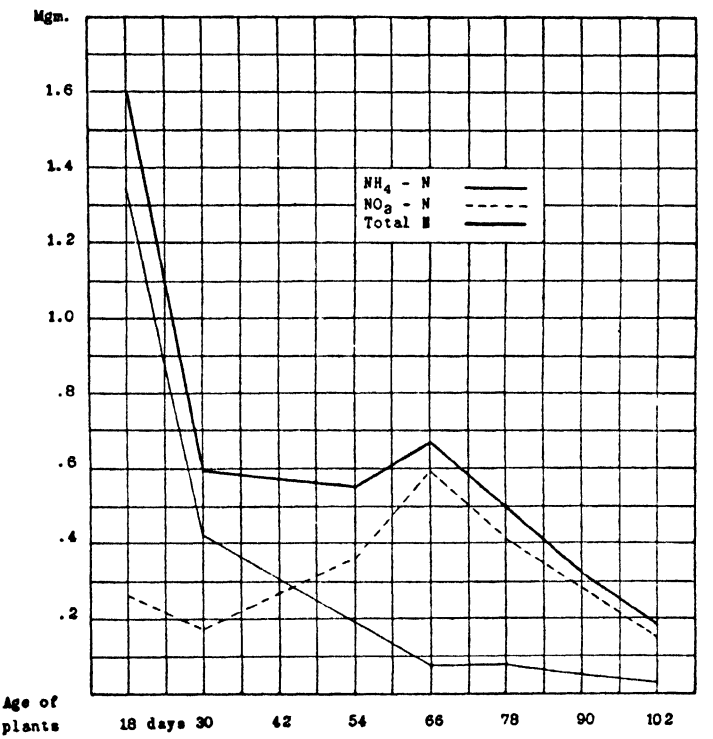

FIG. 5. GRAPHS REPRESENTING THE RATES OF ABSORPTION OF NITROGEN AS NH_4, NO_3 AND TOTAL NITROGEN, IN MILLIGRAMS PER GRAM OF DRY PLANT MATERIAL PER HOUR, AT DIFFERENT PERIODS THROUGHOUT THE LIFE CYCLE OF THE OAT PLANT

proportions. However, in the preliminary tests here reported, approximately the same results were obtained with a solution in which the nitrogen in the two forms is present in quite unequal proportions. No consideration has here been given to the influence of the proportions of the non-nitrogen bearing salts or ions in the culture solution on the rates of nitrogen absorption. What would be the influence of widely different chemical and physical properties of culture media, or widely different sets of environmental conditions, upon the rates of nitrogen absorption from culture solutions, cannot now be stated.

SUMMARY

Quantitative studies were made of the absorption of nitrogen by oat plants from culture solutions containing approximately equal proportions of nitrogen as NH_4 and NO_3, at various stages in the life cycle of the plants. The main results of the investigation may be summarized briefly as follows:

Quantitative analyses show that quantity absorption of nitrogen as NH_4 (milligrams per culture per hour) is highest during the earliest period here investigated, gradually declines, and reaches its minimum point at maturity.

Quantity absorption of nitrogen as NO_3 (milligrams per culture per hour) shows its minimum point at the earliest period investigated, rapidly increases, and reaches a maximum at the blossoming stage, and then rapidly declines to a secondary minimum with approaching maturity.

Quantity absorption of total nitrogen attains a maximum point which corresponds to the point of maximum absorption of nitrogen as NO_3. This occurs during the flowering period.

At no time during the active life cycle was it found that the absorption of nitrogen as NH_4 or as NO_3 entirely ceased.

Rate absorption of nitrogen as NH_4 (milligrams per gram of dry tissue per hour) is at its maximum during the earliest period here investigated, declines rapidly during the early stages of growth, and reaches a very low minimum rate with approaching maturity.

Rate absorption of nitrogen as NO_3 (milligrams per gram of dry tissue per hour) is low during the early stages of development, attains its maximum at the blossoming stage, then declines to a secondary minimum at maturity.

The maximum NH_4-rate of absorption of nitrogen is more than double the maximum NO_3-rate.

Rate absorption of total nitrogen attains a maximum point which corresponds to the point of the maximum NH_4-rate of nitrogen absorption and is determined by the NH_4-rate. A secondary maximum rate occurs at the blossoming stage, corresponds to, and is determined by, the point of the maximum NO_3-rate.

Two periods in the growth cycle occur when the plants show higher activity with respect to the rate of nitrogen absorption (milligrams per gram dry plant material per hour) than they do at other periods: the greatest activity occurring at a very early stage and the second period of high activity during the blossoming stage.

Data are presented to emphasize the importance of some adequate system of continuous flow solution renewal, not only in nutritional studies with plants in culture solutions, but also for the growth of experimental plants in solution or sand cultures.

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LYSIMETER STUDIES: III. THE MOVEMENT AND TRANSLOCATION OF NITROGEN AND ORGANIC CONSTITUENTS IN THE PROFILE OF A PODZOLIC SOIL[1]

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The organic matter and the nitrogen of the soil are intimately related, inasmuch as most of the combined nitrogen, irrespective of the source, is tied up with the organic matter. It is the all important constituent in the nitrogen cycle in nature. It serves as the potential storehouse and accumulator—directly as the organic nitrogenous compounds and indirectly as a source of energy for the microbial flora involved in the process of nitrogen fixation—of the soil's nitrogen resources.

One of the important links in the nitrogen cycle is the process of nitrification with nitrates as the end product. Nitrates are the most mobile nitrogenous constituents and in the balance sheet of nitrogen economy in the soil are very prominent. In the studies on the nitrogen cycle in the soil the nitrates have received the lion's share of attention.

Volumes have been written reporting the investigations on the nitrogen cycle in the soil. A cursory survey of the research projects in the various experiment stations in the United States and elsewhere shows that the nitrogen problem occupies an important place in the research programs of these institutions. Our own, New Jersey Experiment Station, widely known cylinder and plot experiments have been established with the primary view of studying the nitrogen problem, principally one phase of it—denitrification.

And yet this problem is still somewhat of a *terra incognito* and additional information is highly desirable. This is especially true now when our ideas about soils are undergoing a radical change and all soil reactions are being examined in the light of the newer knowledge about the soil profile.

The new lysimeter equipment installed at this station (2) offers an opportunity to study the movement and translocation of organic matter and nitrogen through the undisturbed soil profile. The results which are presented in the following pages seem to give some hitherto unknown and, in a way, unexpected facts.

PERCOLATION OF NITROGEN THROUGH PROFILE

For cropped land, it is generally accepted that the nitrogen losses from the soil in the drainage consist primarily of nitrates. According to Lyon and

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

Bizzell (5, p. 61) "nitrogen in the drainage water is *all* in the form of nitrates." Collison and Mensching (1, p. 40) report that "*almost all* of the nitrogen in the drainage water is in the nitrate form." We shall see presently that this does not, necessarily, hold true for virgin soils.

According to Lyon, Bizzell, Wilson, and Leland (6), the average annual loss of nitrogen in the drainage is 75 pounds for the unplanted tank without lime and 61 pounds for the unplanted tank with lime. Losses of nitrogen have been found to be about 9 to 10 times less from the cropped tanks. The tanks which had a grass crop lost the least amount of nitrogen. A complicating factor in the interpretation of the Cornell lysimeter results is the mode of treatment, namely, the tanks received manure applications which for the period recorded added to the soil 564 pounds of nitrogen per acre. If we assume that all of the nitrogen from the manure disappeared in the drainage as nitrates—an assumption which is unjustified—there is an equivalent of about half of the nitrogen noted above lost from the soil proper.

Collison and Mensching (1) in reporting the results of the lysimeter investigations at the Geneva Experiment Station give the figure of 120.6 pounds of nitrogen per acre lost annually in the drainage from the fallow tanks.

Miller (8) in reporting the results of the lysimeter investigations at Rothamsted gives the figure on the average annual loss of nitrogen from the fallow tank as 30 pounds per acre of which 27 pounds is in the form of nitrates. These figures were substantiated later by Russell and Richards (9).

Many reports of a similar nature on the losses of nitrogen in drainage may be found in the literature concerning lysimeter investigations in Germany and elsewhere, but the three mentioned may suffice to illustrate the difficulties which we encounter when we attempt a correct appraisal of the losses of nitrogen in drainage. Whereas the three stations mentioned show wide variations in their reports on the nitrogen losses, these may not be attributed to differences in soils and meteorological conditions. This is especially true for the Cornell and Geneva stations, where the drainage has been measured from soil material variously prepared and not from the soil body, as pointed out in a former publication (2).

The Rothamsted results are more reliable inasmuch as the soil itself has been preserved in its natural state. Otherwise the conditions have remained similar to those of the other lysimeter installations: the soil is separated from the bulk of the soil body by the tank inclosure, the rainwater must go through the soil column without any chance for return capillary movement from the ground waters and there is no surface run-off.

Tables 1 and 2 present the results on the total and nitrate nitrogen in the drainage obtained from the A_1 horizon for two consecutive years by the new type of lysimeters[2] whereby the conditions are very close to natural.

The first to be noted is that not all of the nitrogen is in the nitrate form.

[2] A full description of the new type of lysimeter equipment is to be found in an earlier publication (2).

In 1929–30 only 74.6 per cent and in 1930–31 about 60 per cent were in the form of nitrates. The rest of the nitrogen is in some other forms. Very little of the nitrogen seems to appear as ammonia, although qualitatively its presence could be demonstrated. For the present the nitrogen besides the nitrates is to be considered as in the organic form.

TABLE 1

Total N and NO_3N percolated through A_1 in 1929–30

(Leachings for year = 21 liters)

LABORATORY NUMBER OF LEACHING ON WHICH DETERMINATIONS WERE MADE	DATE OF COLLECTION	QUANTITY COLLECTED	TOTAL N IN		NO_3–N IN	
			100 cc. of leachings	Total leachings	100 cc. of leachings	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1929					
3	7/30	1.400	0.631	8.831	0.22	3.080
4	8/15	0.755	0.760	5.738		
5	9/9	4.740	0.640	30.336	0.48	22.752
7	10/3	3.440	0.650	22.360	0.50	17.200
8	10/23	0.930	0.660	6.138	0.48	4.460
9	11/5	0.390			0.48	1.870
11	12/20	0.180			0.48	5.660
	1930					
12	2/18	3.435	0.252	8.656	0.16	5.500
15	6/11	2.200	0.310	6.820	0.24	5.280
1. Total			3.903	88.879	3.04	65.802
2. Average per 100 cc.*			0.558	0.526	0.380	0.394
3. Calculated totals†				110.46		82.74
4. Total per acre in grams‡				6,075.3		4,550.7
5. Total per acre in pounds				13.4		10.0

* The average per 100 cc. under the columns "total leachings" was calculated by dividing the total constituents by the respective total leachings on which determinations were made; in the case of total N by 16.9 liters, and in the case of NO_3 by 16.715 liters.

† To obtain the total for all the leachings the average per 100 cc. as calculated was multiplied by the total leachings for the year, 21.0 liters.

‡ To obtain grams per acre the figures from 3 were multiplied by 55,000, since the area of the lysimeter funnel = $\frac{1}{55,000}$ of an acre, and divided by 1,000 (1 gm. = 1,000 mgm.).

The source of the organic form of nitrogen is the A_0 layer which supplies yearly large quantities of organic matter for the process of decomposition and mineralization. In cultivated soils we find the organic matter in the form of stubble, roots, and weed growth, most of which, because of favorable conditions for decomposition, is completely mineralized. This will, perhaps, explain why only 10 per cent of the total nitrogen was not in the form of nitrates in the Rothamsted experiments. Why there was no other form of

nitrogen in the Cornell lysimeters, where large quantities of organic matter in the form of manure were added periodically, is difficult to explain.

Seasonal variations in concentration of nitrogen in the leachings are not so clearly brought out in the data. There is a tendency for a higher concentration of nitrogen compounds toward the end of the summer and in the fall. This is in line with the findings about the mineral constituents in the leachings as brought out in a previous publication (3). The concentration of ni-

TABLE 2

Total N and NO_3 N percolated through A_1 in 1930–31

(Leachings for year = 29.223 liters)

LABORATORY NUMBER OF LEACHING ON WHICH DETERMINATIONS WERE MADE	DATE OF COLLECTION	QUANTITY COLLECTED	TOTAL N IN		NO_3–N IN	
			100 cc. of leachings	Total leachings	100 cc. of leachings	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1930					
16	7/10	1.300	0.423	5.502	0.240	3.120
17	7/23	1.300	0.480	6.240	0.120	1.560
19	8/24	3.350	0.600	20.100	0.140	4.690
23	11/6	1.455	0.693	10.083		
24	11/19	1.875	0.839	15.728		
25	12/30	4.090	0.651	26.626	0.540	22.086
	1931					
26	2/19	4.100	0.353	14.473	0.280	11.480
27	3/9	1.770	0.620	10.974	0.440	7.788
28	3/29	3.305	0.466	15.378	0.360	11.880
29	4/2	0.665			0.240	1.596
30	4/12	0.505			0.180	0.909
33	6/11	1.950	0.480	9.360		
1. Total			5.605	134.464	2.54	65.109
2. Average per 100 cc.*			0.5605	0.549	0.282	0.319
3. Calculated totals†				160.43		93.22
4. Total per acre in grams‡				8,823.7		5,127.1
5. Total per acre in pounds				19.4		11.3

* † ‡ For explanations of footnotes see table 1.

trogen compounds does not seem to decrease much with dilution during any one season. With respect to the nitrates the concentration factor seems to follow the trend of the total nitrogen, although some departures might be pointed out. More data are wanted before anything definite may be said about this point.

Tables 3 and 4 give the data on the total nitrogen and nitrates which percolated through the A_2 horizon, which is located—the bottom of it—at a depth of 42 cm. in the profile.

TABLE 3

Total N and NO_3 percolated through A_2 in 1929–30

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	QUANTITY COLLECTED	TOTAL N IN		NO_3—N IN	
			100 cc. of leachings	Total leachings	100 cc. of leachings	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1929					
1	6/22	0.165				
5	9/9	2.400	0.482	11.568	0.16	3.840
7	10/3	0.210				
11	12/20	0.090				
	1930					
12	2/18	1.265	0.504	6.376	0.32	4.048
15	6/11	1.395			0.40	5.580
1. Total		5.525	0.986	17.944	0.88	13.468
2. Average per 100 cc.*			0.493	0.484	0.293	0.266
3. Calculated totals†				26.74		14.69
4. Total per acre in grams‡						808.00
5. Total per acre in pounds				3.24		1.78

* † ‡ For explanations on footnotes see table 1.

TABLE 4

Total N and NO_3 percolated through A_2 in 1930–31

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	QUANTITY COLLECTED	TOTAL N IN		NO_3—N IN	
			100 cc. of leachings	Total leachings	100 cc of leachings	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1930					
25	12/30	1.870	0.750	14.025	0.45	8.415
	1931					
26	2/19	1.200	0.366	4.392	0.16	1.920
27	3/9	0.250			0.32	0.800
28	3/30	0.910			0.18	1.638
34	6/18	0.045				
1. Total		4.275	1.116	18.417	1.11	12 773
2. Average per 100 cc.*			0.558	0.600	0.28	0.302
3. Calculated totals†				25.55		12.91
4. Total per acre in grams‡				1,405.3		710.1
5. Total per acre in pounds				3.09		1.56

* † ‡ For explanations on footnotes see table 1.

The first to be noted is that out of 13.4 pounds of nitrogen which leached through A_1 in 1929–30 only 3.24 pounds, or 24.1 per cent, percolated through A_2. It will be recalled (2) that in the corresponding year the leachings from A_2 amounted to 25 per cent of those from A_1. Of the 19.4 pounds of nitrogen which leached through A_1 in 1930–31 only 3.09 pounds, or 15.9 per cent, percolated through A_2. And during this year the leachings from A_2 amounted to 14.6 per cent of those from A_1. Apparently there is a certain correlation between the total nitrogen compounds leached from A_2 and the total leachings.

Another point to be noted in the data on the nitrogen percolation through A_2 is that in both years the amount of nitrate nitrogen with respect to the totals is about the same: a little over 50 per cent. If we compare the forms of nitrogen in the A_1 and A_2 we note that the nitrate nitrogen drops in A_2. Naturally this means that the other forms of nitrogen, especially organic, are on the increase.

The decrease in nitrates in the leachings of A_2 is readily accounted for. During the summer very few leachings appear from A_2; they are retained there and utilized by the growing plants. Most of the leachings from A_2 appear in the winter when the nitrate content drops as a result of the slowing down of the nitrification process.

The data on the leachings from A_2 are scant because there are usually not enough leachings to make total nitrogen analyses. More data are wanted to corroborate the conclusions presented in the foregoing discussion.

ORGANIC MATTER AND NITROGEN RELATIONSHIPS IN LEACHINGS

The movement of organic matter in the soil profile is of paramount importance in elucidating the processes of soil formation. It is generally recognized that it is the organic matter that is responsible for the movement of the sesquioxides and other mineral constituents, especially in the podzols.

The data on the total solids, loss on ignition, and the estimated 10 percent water of hydration (this figure is only an approximation, as it was calculated from the probable water of hydration in the mineral residue) furnish the information on the organic matter in the leachings.

In tables 5 and 6 the data on the total solids and loss on ignition are confined only to the leachings on which nitrogen determinations were made. A complete record of these was presented in a former publication (3). It was found that in 1929–30 the loss on ignition, calculated on the acre basis, was 139.6 pounds, or 125.64 pounds of organic matter percolated through A_1. In 1930–31 the loss on ignition was 258.4 pounds, or 232.56 pounds of organic matter percolated through A_1. For the corresponding years the organic matter in the leachings of A_2 was 19.69 and 13.32 pounds respectively. Thus more organic matter was leached from A_2 in 1929–30 than in 1930–31, notwithstanding the fact that less organic matter reached it from A_1. The converse is true for the organic matter from A_2 in 1930–31.

An examination of the quantities of leachings from A_2 for the 2 years under

consideration shows that in 1929–30 more appeared than in 1930–31 —5.525 and 4.275 liters respectively—even though a smaller quantity of leachings reached A_2 from A_1 in that year—21 in 1929–30 against 29.2 liters in 1930–31.

TABLE 5

Carbon and nitrogen relationships in leachings from A_1

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	LEACHINGS	TOTAL SOLIDS AT 105°C.	LOSS ON IGNITION		TOTAL CARBON*	ORGANIC N†	C N RATIO	N IN ORGANIC MATTER‖
		liters	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*		*per cent*
				1929–30					
	1929								
3	7/30	1.400	289.80	142.24	49.00	74.24	5.751	12.9	4.5
4	8/15	0.755	113.25	43.79	38.6	22.86	2.866‡	8.0	7.4
5	9/9	4.740	611.46	271.13	44.3	141.53	7.584	18.6	3.1
7	10/3	3.440	509.80	181.97	35.6	94.99	5.160	18.4	3.1
8	10/23	0.930	189.72	66.96	35.2	34.95	1.678	20.8	2.8
	1930								
12	2/18	3.435	378.54	78.49	20.7	40.97	3.156	13.0	4.4
15	6/11	2.200	300.96	133.32	44.2	69.59	1.540	45.0	1.3
				1930–31					
	1930								
16	7/10	1.300	245.70	161.42	65.6	84.26	2.38	35.4	1.6
17	7/23	1.300	158.60	91.78	57.8	47.91	4.68	10.2	5.6
19	8/24	3.350	739.68	404.68	54.7	211.24	15.41	13.7	4.2
23	11/6	1.455	480.73	218.25	45.3	113.93	5.44†	20.9	2.7
24	11/19	1.875	571.12	190.69	33.3	99.54	9.75†	10.2	5.6
25	12/30	4.090	503.07	204.45	40.6	106.73	4.54	23.5	2.4
	1931								
26	2/19	4.100	434.60	169.74	39.0	88.61	2.99	29.6	1.9
27	3/9	1.770	180.19	64.61	35.8	33.73	3.19	10.6	5.5
28	3/29	3.305	311.33	115.68	37.1	60.38	3.50	17.2	3.4
33	6/11	1.950	308.10	187.20	60.7	97.72	3.14	31.1	1.8

* The figures on the total carbon were obtained as follows: On the total ignition loss of solids 10 per cent was allowed for water of hydration and the rest was considered as organic matter. By taking the generally accepted 58 per cent of C in organic matter, the total C was calculated.

† Organic N was calculated by subtracting the NO_3–N from the figures on the total N.

‡ The NO_3–N on this sample was calculated by taking the average NO_3 for the year.

‖ The nitrogen in organic matter was calculated as follows: The organic matter was figured out as shown in footnote* and from these and the figures on the organic N the per cent of N in the organic matter was obtained.

The explanation for this phenomenon was discussed in the first paper of this series (2). It is the state of the colloids in the soil that controls the percolation rather than the total volume of rainfall or leachings.

The average percentage loss on ignition of the solids from A_1 was higher in 1930–31 than in 1929–30, about 46 and 38 per cent respectively. This, of course, is due to the higher organic matter content in the leachings of 1930–31. As a matter of fact, the concentration of organic matter was also higher, 15.9 and 14.21 mgm. of solids per 100 cc. of leachings in 1930–31 and 1929–30 respectively. The cause of such a higher concentration is, of course, difficult to explain. Apparently the environmental conditions for microbial activities in the A_1 horizon were more favorable in 1930–31 than in 1929–30. Unfortunately practically nothing has been done on the differential behavior of microörganisms in the various horizons of the soil profile.

TABLE 6

Carbon and nitrogen relationships in leachings from A_2

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	LEACHINGS	TOTAL SOLIDS AT 105°C.	LOSS ON IGNITION		TOTAL CARBON*	ORGANIC N†	C.N RATIO	N IN ORGANIC MATTER
		liters	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*		*per cent*
				1929–30					
	1929								
5	9/9	2.400	242.40	69.60	28.7	36.33	7.728	4.7	12.3
	1930								
12	2/18	1.265	146.7	45.4	30.9	23.20	2.328	10.0	5.7
15	6/11	1.395	113.27	54.68	48.2	28.54	1.172†	24.3	2.4
				1930–31					
	1930								
25	12/30	1.870	204.20	71.06	34.3	37.09	5.610	6.6	8.7
	1931								
26	2/19	1.200	89.76	24.96	27.8	13.03	2.472	5.2	10.0
28	3/30	0.910	65.52	18.29	27.9	9.55	3.820	2.5	23.0

* † ‡ See footnotes in table 5.

The loss on ignition of the solids from A_2 was higher in 1929–30 than in 1930–31, 34.8 and 31.7 per cent respectively. [These figures are taken from the second paper of this series (3) where all the leachings are recorded.] Again the higher organic matter content is responsible for the higher loss on ignition. It is to be noted that the concentration of organic matter in the leachings of A_2 dropped considerably when compared with the leachings from A_1: 77 per cent in 1929–30 and 82 per cent in 1930–31.

There is considerable difference in the chemical make-up of the organic matter of the various horizons in the profile. This is very lucidly brought out in the data on the C:N ratios of the organic matter in the leachings from A_1 and A_2. During both years the ratio was high for the organic matter in the leachings from A_1 and low for A_2. But even within each horizon there is a

difference in the organic matter from the leachings from year to year. If we disregard the extremely high C:N ratios, especially leaching number 15—a procedure justified by tests with statistical methods—and average the others we find 15.3 and 18.5 for A_1 and 7.3 and 4.8 for A_2 respectively during the 2 years under consideration. A higher C:N ratio indicates a type of organic matter low in protein content, hence with a low ash content and high loss on ignition. This is what apparently happened with the organic matter in the leachings from A_1 in 1930–31 and from A_2 in 1929–30: the organic matter contained constituents of a lower protein nature than the organic matter in the leachings from A_1 in 1929–30 and from A_2 in 1930–31, and hence the difference in loss on ignition.

An examination of the nitrogen content of the organic matter, as presented in the data of tables 5 and 6, shows that it follows the loss on ignition: the higher the loss on ignition the lower the nitrogen content and vice versa. And this is to be noted: in A_2 the nitrogen content of the organic matter is by far greater than in A_1—4.2 and 3.5 for A_1 and 9 and 13.9 for A_2 in 1929–30 and 1930–31 respectively. Of course these wide differences in nitrogen content are due to differences in the type of organic matter.

The data on the organic matter in the leachings of the A_1 and A_2 horizons and on the nitrogen content of it offer a clue to the phenomenon noted in a different connection—in a study of the podzolization process (4)—namely, that the B horizon becomes enriched with organic matter of a high nitrogen content. The extremely high N content—in one case as much as 23 per cent—in the organic matter of the leachings from A_2 calls for an explanation. There is the probability that fresh animal excreta had been deposited at the time of that leaching. And we know that such highly nitrogenous compounds as urea are found in animal excreta. An almost similar amount of N—22 per cent—in humus substances of a podzol soil has been reported by Weis (10, p. 192).

GENERAL DISCUSSION

An important point which the foregoing pages attempted to bring out is that in forest soils the losses of nitrogen either in the form of nitrates or in any other form are not so great as those reported for cultivated soils. The lingering of the leachings in the soil profile and the relatively small quantities of leachings available for the ground waters, as pointed out in the first paper of this series (2), have indicated such a course. Actual analyses of the leachings show that even if we should consider that the nitrogen which passes the A_2 horizon finds its way to the ground waters (it is very probable that a good share of it remains long enough in the B horizon to be taken up by the plants), there are compensating factors which more than cancel the losses. One need not go into the aspect of nitrogen fixation, which is fairly well established, although no definite quantitative value may be assigned to this source. The nitrogen supplied by the rainfall alone is quite sufficient to offset the

losses by leaching. If it were not for the N dissolved in the rainwater and that fixed by microörganisms, where and how could the soil— I have in mind the virgin soils and those cultivated soils which reached a definite nitrogen minimum—maintain its nitrogen supply?

Analyses of rainwater show that very definite quantities of nitrogen are added annually to the soil through this medium. According to Miller (7), who reviewed the subject very thoroughly (125 references are given) up to his time, and Wilson (11), who reviewed the subject up to a later period, the annual average addition of nitrogen to the soil by rainfall does not fall below the figure of 5 pounds per acre. The data in tables 3 and 4 show that only a little more than 3 pounds of nitrogen per acre goes through the A_2 horizon, a figure below the amount supplied by the rainfall.

Data on the quantity of leachings from horizons B and C during these two years and on the number of times leachings appeared from these horizons are so scant that no definite conclusions can be reached. In all, only 1 per cent of the leachings passed through the B horizon and about 2 per cent through C. For an explanation of this anomalous phenomenon the reader is referred to the first paper of this series (2).

From the meager data on hand there is evidence that an appreciable amount of nitrates is retained in the B horizon. Thus leachings from C give, as a rule, a lower nitrate content than the leachings from any other horizon. The quantities of leachings obtained from B and C were not sufficient for nitrogen determinations, but from theoretical considerations and tests on the organic colloid fraction of a podzol soil, it seems (4) that in these horizons the colloids are highly dispersed and the nitrogen content of the organic matter is high.

An important element in the process of moisture percolation through the profile and in all the reactions accompanying it is, of course, the rainfall. It is to be recalled that during the 2 years under consideration it was below normal, and although the percolation is not entirely dependent on the total rainfall, as shown in the first paper of this series (2), it remains to be seen what the results will be when the rainfall shall be normal or above normal.

SUMMARY

A discussion on the data of lysimeter investigations, as exemplified by the Cornell, Geneva, and Rothamsted reports, on the nitrogen losses from soils is presented showing wide variations.

The data on the nitrogen in the leachings from the new type of lysimeters show that in forest soils the nitrogen losses are not entirely in the form of nitrates. Only 50 to 75 per cent of the nitrogen appears as nitrates through the A_1 horizon and still less through the A_2 horizon.

Only 3.24 pounds of total nitrogen percolated through A_2 in 1929–30 and only 3.09 pounds in 1930–31. Thus only small amounts of total nitrogen are lost.

Data on the organic matter content in the leachings show that the type

of organic matter varies but slightly within each horizon from year to year. There is, however, considerable difference between the organic matter in the leachings of the A_1 and A_2 horizons.

The differences in the organic matter from the various horizons are easily noted from the data on the C:N ratios and the nitrogen content of the organic matter. The ratio is high in A_1 and low in A_2, giving a high nitrogen content to the organic matter in A_2. This offers a clue to the observed phenomenon of a high nitrogen content in the organic matter of the B horizon.

It is pointed out that the scant leachings from B and C prevent the evaluation of the true loss of nitrogen from the soil, but even if one accepts the losses from A_2 as typical—an assumption not justified—they are small, and the addition of nitrogen by the rainwater more than offsets these losses. Appreciable amounts of nitrates are retained by the B horizon and for this reason the nitrate content in the leachings from C is, as a rule, low.

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ERRATUM

Estimation of plant available phosphate in soil

By P. L. Hibbard, SOIL SCIENCE, Vol XXXV

Page 22: Line 8 of paragraph 2, *0 5 N* H_2SO_4 should read *0 05 N* H_2SO_4

THE DIFFUSION OF CARBON DIOXIDE THROUGH SOILS[1]

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The importance of carbon dioxide production in soils has long been recognized, and various methods have been proposed for its determination. The first determinations involved measurements of the concentration of carbon dioxide in the soil air. It was assumed that a high concentration of carbon dioxide was synonymous to a high rate of production. However, it was soon found that although this may be true generally, it is not necessarily true under all conditions. The carbon dioxide produced may accumulate in one soil to a greater extent than in another even though the rate of production is the same in both cases. In other words, the concentration of carbon dioxide in the soil air, or its accumulation in the pore space of the soil, is a function not only of the rate of production but also of the rate of escape. Carbon dioxide produced in the soil may diffuse in any direction from the place where it is produced, it may go into solution, or it may be fixed in the soil.

The evolution of carbon dioxide from small samples of soils in the laboratory was next considered a measure of the rate of production. The desirability of measurements of rate of production in soils *in situ* was early recognized, and certain methods were adapted for that purpose However, the evolution of carbon dioxide from a small sample of soil, although far from giving an accurate measure of the rate of carbon dioxide production, is perhaps still the most reliable method for its determination.

Lundegardh (3) defined *soil respiration* as the amount of carbon dioxide in grams evolved from 1 sq.m. of soil *in situ* in 1 hour, and the rate of respiration was considered a measure of the rate of production. However, Smith and Brown (5, 6) found that soil respiration is not a simple diffusion of carbon dioxide from the soil and, therefore, is not an accurate measure of the rate of production.

Buckingham (1) made a study of the speed of diffusion of air and carbon dioxide into each other through the soil. He concluded that the speed of diffusion of carbon dioxide through the soil is proportional to the square of the percentage of free pore-space, and that the texture, structure, and moisture content do not influence it to any great extent. The mean diffusion constant for oxygen and carbon dioxide diffusing through 79 per cent nitrogen was computed and it was assumed that the same relation holds for the diffusion of carbon dioxide through the soil. Then, by means of this diffusion constant, the porosity of the soil, and the concentration of carbon dioxide in the soil air, the rate of production was calculated.

Buckingham recognized numerous sources of error in the determination of his diffusion constant, such as the thickness of soil, the analyses of the gases, and the regulation of pressures.

The purpose of the work reported here was to determine the relation between the rate of diffusion of carbon dioxide and the porosity of the soil, avoiding, if possible, some of the sources of error recognized by Buckingham and to ascertain what relation, if any, exists between the concentration of carbon dioxide in the soil, the rate of diffusion, and the respiration of the soil. The term "soil respiration" is used here as defined by Lundegardh (3).

[1] Journal Paper No. *B71* of the Iowa Agricultural Experiment Station.

EXPERIMENTAL

It was concluded that if the difficulties recognized by Buckingham and others were to be avoided, it would be necessary to maintain different partial pressures of carbon dioxide on two faces of a soil column and the same total pressure on each side, to obtain the column of soil as it exists in the field without disturbing its structure, and to make the analysis of the diffused gas directly.

Hannén (2) studied the rate of diffusion of carbon dioxide through soils of different texture. He placed a column of soil in one cylinder above another of the same size which was filled with pure carbon dioxide. After 10 hours the amount of carbon dioxide remaining in the lower cylinder was determined and the amount which had disappeared was assumed to have escaped by diffusion through the soil. In the work reported here, an effort was made to measure directly the actual amount of carbon dioxide diffusing through the soil.

An attempt was made to obtain the column of soil with structure unchanged by taking the sample from the field in a special apparatus. A brass cylinder 3 inches in diameter and 10 inches long (plate 1, fig. 1) was used for this purpose. The brass cylinder was made up in three sections and joined together by ground, flanged joints which fit tightly. The lower section was 1½ inches high and was sharpened to a cutting edge on the lower end. The top section was 2½ inches long and was fitted with a piece of solid cast iron for driving the cylinder into the ground. After the cylinder was driven into the ground it was dug out with a spade and carried into the laboratory, the top and bottom sections were removed, and the soil was sheared off smooth. The cylinder with the core of soil formed the center portion of the diffusion apparatus (plate 1, fig. 2). The lower chamber was 3 inches high and had two vents, an inlet tube for the gas mixture at the bottom and an outlet near the top of the section, or just below the core of soil, which was left open to the atmosphere. The top section of the apparatus was a chamber 3 inches high with two vents, one being at the bottom of the section, or just above the core of soil, to which a small U-tube manometer filled with water was attached, the other opening being at the top and used for taking samples of the gas for analysis. This section was also fitted with a stirrer to mix the gas before sampling. The apparatus was jointed together and tightly clamped by means of bolts with wing-nuts and a wooden clamp. The joints were coated with hot paraffin before being joined.

The concentration of carbon dioxide in the chamber above the soil was determined. A mixture of air and carbon dioxide of definite carbon dioxide content was passed slowly into the bottom chamber of the apparatus while the other opening near the bottom of the core of the soil was left open. Thus, air of a definite concentration of carbon dioxide was maintained on the lower face of the soil at atmospheric pressure. The air in the top cylinder was stirred, and samples were taken at intervals for analysis. The coefficient of diffusion, designated by K, is defined as the number of cubic centimeter of car-

bon dioxide at 0°C. and 760 mm. of mercury passing through 1 sq. cm. area of cross-section of soil 1 cm. thick per second when the partial pressures of CO_2 on the two faces of the soil differ by 1 mm. of mercury and the total pressure is 760 mm. The constant was calculated from the data according to the equation:

$$K = \frac{X\,H}{(C_1 - C_2)\,A\,t\,760}$$

in which X = The volume of carbon dioxide passing through the core of soil (0°C. 760 mm.).
H = The height of the column of soil in cm.
C_1 = The concentration of carbon dioxide on the lower face of the column of soil.
C_2 = The logarithmic average of initial and final concentrations of carbon dioxide on the upper face of the column of soil.
A = The area of cross section of soil, cm^2.
t = Time in seconds.

Since the diffusion of carbon dioxide through this column of soil depends upon the difference in concentration in carbon dioxide at the two faces of the core of soil and the diffusing gas is accumulated in the top chamber of the apparatus, the rate of diffusion will be decreased as the concentration of carbon dioxide in the chamber above the soil increases. The concentration of carbon dioxide in the chamber above the soil is constantly changing, hence initial and final concentrations were determined, and the logarithmic average was taken as the concentration for calculating the average rate of diffusion.

It was noted after the first experiment was performed, that carbon dioxide was being produced in the column of soil. This carbon dioxide produced during the experiment interfered with the determination and all attempts at sterilization of the soil without altering its structure failed. An attempt was made to determine the amount of carbon dioxide evolved during the experiment and correct for it in the calculation. Air was drawn through the soil by means of a vacuum pump at the rate of 10 liters per hour for 30 minutes, after which the concentration of carbon dioxide was determined in the chamber above the soil. A micro-Haldane gas analysis apparatus was used for the analysis. The concentration of carbon dioxide was determined again at 30-minute intervals for 2 hours and the amount of carbon dioxide evolved calculated. A constant rate of production being assumed, the amount of carbon dioxide produced during the experiment was subtracted from the total found in the chamber above the soil at the end of the experiment. The difference represented the amount passing through the soil by diffusion.

The porosity of the soil was determined as follows:

Weight of cylinder + moist soil	1,800 gm.
Weight of cylinder	870 gm.
Weight of moist soil	930 gm.
Weight of moisture	167 gm.
Weight of dry soil	763 gm.

Sp. Gr. of soil	2.24
Volume of soil	340 cc.
Volume of cylinder	577 cc.

$$\text{Porosity} = \frac{577 - (340 + 167)}{577} = 0.121$$

Diffusion of carbon dioxide through moist soil

A column of Carrington loam was taken from the Agronomy Farm and the rate of diffusion of carbon dioxide through the soil determined according to the

TABLE 1

Diffusion of carbon dioxide through moist soil

LENGTH OF DIFFUSION PERIOD	PERCENTAGE CO_2			PERCENTAGE INCREASE		$K \times 10^{-4}$
	Below soil	Above soil		Due to evolution of CO_2	Due to diffusion of CO_2	
		In beginning	After diffusion			
hour:minutes						
1:30	1.28	0.033	0 116	0.023	0 060	3.81
2:00	1.28	0.033	0.165	0.031	0.101	5.15
1:00	0.99	0.025	0.059	0.034	0 000	0.00
2:00	0.99	0.025	0.150	0.068	0.057	4.77
3:30	0.99	0.025	0.276	0.119	0.133	7.29
1:00	1.03	0.025	0.050	0.029	−0.004	0.00
2:00	1.03	0.025	0.150	0.058	0.067	5.12
1:00	1.22	0.024	0.082	0 026	0.032	3.31
2:00	1.22	0.024	0.173	0.052	0.097	5.35
4:00	1.22	0.024	0.346	0.104	0.218	5.29
1:00	0.536	0.040	0.041	0.024	−0.023	
2:30	0.536	0.040	0.208	0.061	0.107	21.8
1:00	1.545	0.049	0.049	0.024	−0.025	0.00
2:30	1.545	0 049	0 155	0.061	0.045	1.10
3:00	1.545	0.049	0.237	0.072	0.117	2.81
4:30	1.545	0.049	0.357	0.108	0.200	4.61

procedure outlined in the foregoing. Sixteen determinations were made, the length of the diffusion period varying from 1 to 4½ hours, and the diffusion constant was calculated. The results obtained appear in table 1.

The data in the table show that the average rate of diffusion increased with the length of diffusion time in all cases, except one, where it was approximately the same after 4 hours as after 2 hours. The factors influencing the rate of diffusion were the same for every determination, at least as nearly as it was

possible to maintain them under the conditions of the experiment. Thus, it would seem that the correction factor applied did not account for the amount of carbon dioxide produced in the soil during the period of the experiment. Thus, whereas the determination of the amount of carbon dioxide produced was made as accurately as possible, no account was taken of any of the gas passing into solution, adsorbed by the soil, or fixed in the soil. It appears

TABLE 2

Diffusion of carbon dioxide through air-dry soil ($K \times 10^{-7}$)

NUMBER OF DETERMINATIONS	POROSITY					
	0.364	0.426	0.512	0 518	0 625	0.645
1	1 99	1 91	1.72	1 89	2 58	2 90
2	3.11	1.79	1 92	2 99	3 00	3 19
3	1.83	0.89	2 16	3 26	2 66	2 29
4	2.33	1 01	3.38	2 81	3 43	2.53
5	2.25	2 36	1 73	1 54	2 41	2.35
6	2.75	2 14	2.94	2 67	2.97	3 46
7	2.25	3 98	2 85	3 50	2 67	3 66
8	2.55	1 13	2 89	1 77	2 21	3 06
9	2 60	2 72	2.26	2 49	0 77	4 05
10	2 60	2.13	2 85	2 73	2 76	2.29
11	1.10	2.25	2 57	3 55	2.78	2 31
12	1.65	3 25	3.17	3 35	2 14	3 87
13	2.44	2 62	....	...	1 08	2 77
14	....	2 68	....	.. .	2 15	3.54
15	....	3 15	....	. ..	3 46	3 99
16	....	3 09	. .	....	2.18	5 05
17	. ..	2.67	....	.. .	2 24	3 73
18	. ..	2 55	....	....	2 95	2 97
19	....	2.28	. ..	....	1 77	3.70
20	..	2.71	..	.	1 92	4 53
21	....	2 61	.. .	. ..	2 08	3 24
22	. ..	....	....	....	1 96	....
23	.. .	. ..	...	....	2.88	. ..
24	. ..	....	....	....	2 52	....
25	. ..	. .	...	.. .	3 19	....
Means....	2 265	2.377	2.537	2 713	2 430	3 309

that some of this carbon dioxide which cannot be accounted for may influence the rate of diffusion considerably. It is possible under conditions of rapid production of carbon dioxide to increase the concentration of carbon dioxide within the core of soil to a point where it would actually diffuse in both directions, that is, into both the upper and the lower chambers of the apparatus. The procedure was finally modified to consist of a determination of the average rate of diffusion through air-dry soil.

Diffusion of carbon dioxide through air-dry soil

A ½-inch section of cylinder with fine-mesh brass screen was placed between the bottom chamber and the middle section of the cylinder. The air-dry soil was placed in this section and tamped lightly to compact the soil, and the apparatus was assembled. The porosity was then determined as in the previous work.

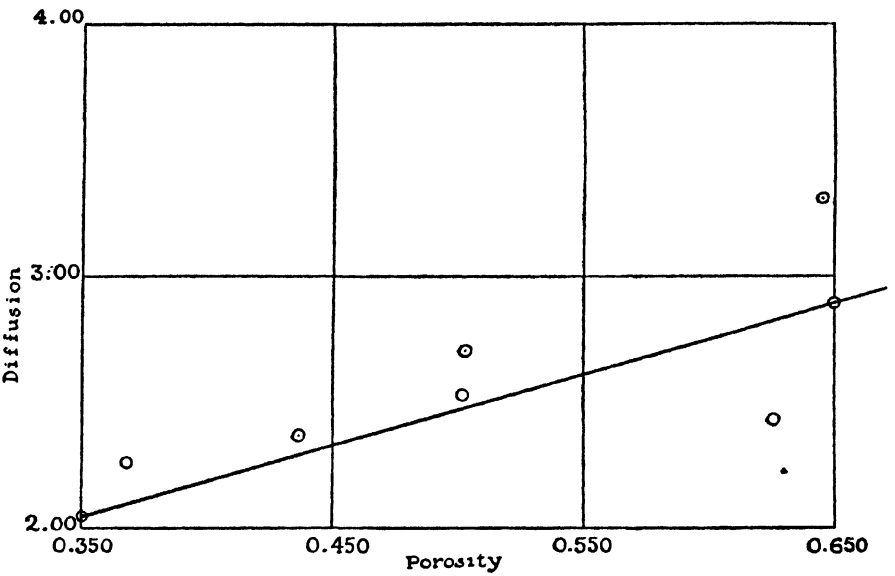

FIG. 1. REGRESSION LINE SHOWING THE RELATION OF RATE OF DIFFUSION OF CARBON DIOXIDE THROUGH AIR-DRY SOIL TO THE POROSITY OF THE SOIL

A large number of determinations on the rate of diffusion of carbon dioxide were made at 30-minute intervals. Six soils of different porosity were used. The results obtained are presented in table 2.

The data in the table show considerable variation within the porosity groups. However, the means of these groups, when tested by the method of analysis of variance[2] were found to vary significantly among themselves. That is, the average speed of diffusion of carbon dioxide through air-dry soil was found to be definitely related to the porosity of the soil.

Furthermore, it appears from the means in the table and from figure 1 that there is a trend toward higher rates of diffusion as the porosity increases. To test the significance of this trend, the correlation between rates of diffusion and porosity was computed and found to be 0.31, a highly significant value. The regression line, plotted in figure 1, has the equation,

$$\text{Dif.} \times 10^7 = 2.297 \text{ (porosity)} + 1.402$$

[2] Prof. G. W. Snedecor, statistical laboratory, Iowa State College, Ames, Iowa.

The regression coefficient indicates an average increase of 2.297×10^{-7} cc. carbon dioxide per unit increase in porosity, or 2.297×10^{-9} cc. for each per cent increase in pore space.

The diffusion of carbon dioxide through soils in situ and the relation to production and respiration

The average rate of diffusion of carbon dioxide through air-dry soil was found to be definitely related to the porosity of the soil. Inasmuch as the porosity of the soil is greatly altered by the structure of the soil and its moisture content, the rate of diffusion under field conditions would be expected to be very different from the average rate obtained with air-dry soil. Studies were made on the rate of diffusion of carbon dioxide through Carrington loam *in situ*. A respiration bell similar to the one used by Lundegardh (3) was used to collect the carbon dioxide evolved from the surface of a given area of soil. A tube (7) was placed in the soil at a depth of 15 cm. from which samples of soil air were taken for a determination of the concentration of carbon dioxide. The percentage of carbon dioxide in the bell air was determined in the beginning of the experiment and again after 20 to 30 minutes. The amount of carbon dioxide evolved was expressed as cubic centimeters at 25°C. and 760 mm. pressure. The rate of diffusion was calculated according to the equation:

$$\text{Diffusion} = \frac{R \cdot H}{(C_1 - C_2) \cdot A \cdot t \cdot 760}$$

in which R = The number of cc. carbon dioxide at 25°C. and 760 mm.
H = Depth of sample of soil air taken for analysis.
C_1 = Percentage of carbon dioxide in soil air at H.
C_2 = Logarithmic average percentage of carbon dioxide in the bell air from the beginning to the end of the experiment.
A = Area of soil under the bell.
t = Time in seconds.

The diffusion values obtained under these conditions would not, of course, represent the rate of diffusion, since carbon dioxide is being produced in the soil while the measurements are being made. The results may, however, be of value as an indicator of soil moisture conditions, aeration, and, in fallow soils, as an index of microbiological activity.

The respiration and the rate of production of carbon dioxide in this soil were calculated from the measurements made, and the results were expressed as milligrams carbon dioxide per square meter per hour. The rate of production was calculated by assuming the simple relation, production = concentration (CO_2 pressure gradient per centimeter) times diffusion. The coefficient of diffusion being that for air-dry Carrington loam. The average moisture content was about 15 per cent and the free pore-space was 0.350. The results obtained are presented in table 3.

The data in the table show a considerable variation in the percentage of carbon dioxide in the soil air at a depth of 15 cm. from August 3 to September

10. The respiration rate varied widely during the same period but showed no apparent relation to the concentration of carbon dioxide in the soil air. The diffusion value obtained by calculation from the concentration of carbon dioxide and the respiration, a resultant of production, solution, and diffusion, is perhaps a more reliable index of microbiological action than either concentration or respiration alone. It is, however, difficult to obtain representative samples of the air under the bell for analysis. The inaccuracies in the measurement of the amount of carbon dioxide evolved from the soil are reflected in the diffusion value. Furthermore, the numbers mean nothing in so far as showing anything about the rate of diffusion of carbon dioxide through the soil is concerned.

The rate of production as calculated from the concentration of carbon dioxide in the soil air was much lower than the respiration. These results show the

TABLE 3

The relation of concentration of CO_2 in soil air to rate of production, diffusion, and respiration

DATE (1931)	PERCENTAGE CO_2 IN SOIL AIR AT 15 CM. DEPTH	DIFFUSION $\times 10^{-6}$	RESPIRATION (MGM. CO_2 PER SQ.M. PER HR.)	RATE OF CO_2 PRODUCTION (MGM. PER SQ.M. PER HR.)
August 3	1 00	3 358	81 75	3 94
August 5	0 89	2 865	66 83	3.77
August 6	0 87	2.577	49 89	3.35
August 7*	0 88	2.639	53.25	3 21
August 10	0.54	0 719	9 37	2.00
August 21	0.60	2.535	39.40	2.19
August 24	0 93	32.750	826 70	3.68
August 25	0 88	14 100	296 30	2.93
August 27	0 63	3.560	52 75	2 42
September 2	0 66	2 957	52 84	2 93
September 10	0 30	8 832	14 66	1 16

* Raining.

same variation as the percentage of carbon dioxide in the soil air, are based on static or constant conditions which are not obtainable in the soil, and cannot, therefore, be regarded as a reliable measure of the rate of production of carbon dioxide in the soil.

SUMMARY AND CONCLUSIONS

The diffusion of carbon dioxide through moist soil was found to be so complicated by the production of carbon dioxide in the soil while the measurements were being carried out that an accurate determination of the rate of diffusion could not be made. This, no doubt, explains why Buckingham found diffusion to be an exponential function of porosity and concluded that diffusion was not influenced to any great extent by moisture, texture, and structure. These factors do influence the rate of diffusion, at least in so far as they affect porosity,

since diffusion is a function of porosity. An effort was made to estimate the amount of carbon dioxide produced during the experiment and correct for it in the calculation of the diffusion coefficient. Finally, however, this variable was eliminated by using air-dry soil. More than 100 experiments were made, using air-dry soils differing in porosity. It was concluded from the data obtained that the rate of diffusion of carbon dioxide through air-dry soil is a linear function of porosity of the soil within the limits of porosity studied. This does not mean, however, that at zero porosity, that is, soil saturated with water, that there would be no diffusion of carbon dioxide through the soil; nor that at 100 per cent porosity, that is, free diffusion, this relation would hold.

The diffusion of carbon dioxide through Carrington loam *in situ* was determined 11 times over a period of about 5 weeks. The results obtained varied from 2.535×10^{-8} to 32.750×10^{-8}. Since the method is subject to certain inaccuracies, caution must be observed in the interpretation of the data. However, since it is a resultant of many (4) forces some of which are related to the production of carbon dioxide in the soil, it would seem to be a better index of the amount of carbon dioxide produced in soils *in situ* than either the concentration of carbon dioxide in the soil air or the evolution of carbon dioxide from the soil alone.

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PLATE 1

CARBON DIOXIDE DIFFUSION APPARATUS

FIG. 1. Parts of diffusion apparatus

FIG. 2. Diffusion apparatus assembled

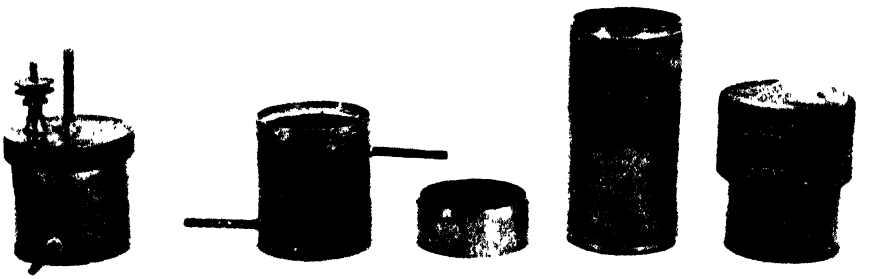

FIG. 1

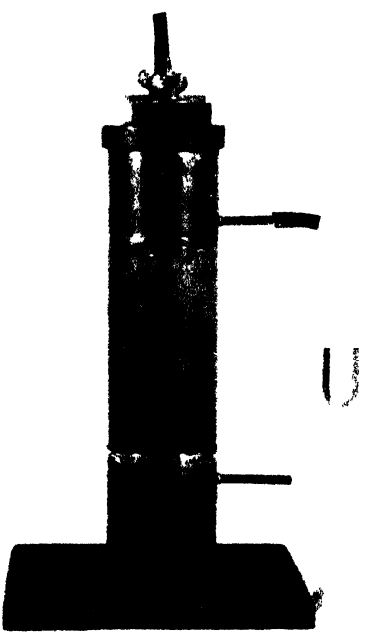

FIG. 2

THE IODINE CONTENT OF THE SOIL IN KENTUCKY[1]

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Kentucky Agricultural Experiment Station

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The importance of iodine in the metabolism of animals was established only recently through the researches of Baumann (1) who, in 1895, proved that iodine is localized in the thyroid gland. In 1915, Kendall (4) demonstrated that the thyroid gland secretes the hormone thyroxine, which contains 65 per cent of iodine. That the presence of a small but adequate quantity of iodine in the organism is necessary to the physiological well-being of man, livestock, and plants is now widely recognized. The iodine contained in the bodies of animals is absorbed from the food and water ingested; that in plants, if we exclude fertilizers, comes from the soil. It is therefore of fundamental importance in the sciences of agriculture, medicine, and nutrition, to ascertain whether the soil of any region which produces foods is adequately supplied with iodine. Thus far only a few investigators have determined iodine in soils, and these investigations, for the most part, were carried on in foreign countries.

REVIEW OF PREVIOUS WORK

About 1850, Chatin (3) first demonstrated that small quantities of iodine are widely distributed in nature. He also pointed out that iodine is not equally distributed in natural waters, rocks, and soils and that endemic goiter is associated with certain areas of soils which are low or deficient in iodine, in his country. Chatin's theory, that iodine-deficient foods and waters are a cause of endemic goiter, was not accepted by his contemporaries at the French Academy of Science, where his paper was presented; however, the researches of a number of investigators in recent years have sustained its correctness.

Benson and Carter (2) published analyses showing the iodine content of soils from Western Australia, New Zealand, and adjacent islands. The maximum iodine content of six soils from Australia was 9.6 p.p.m.: the minimum, 0.7 p.p.m., and the average, 3.53 p.p.m. Five samples of soil from islands near by, derived from basaltic rocks, contained about 10 times as much iodine as the average of the soils from Australia.

Shore and Andrew (7) reported the iodine content of 65 soils collected in several districts of the North Island and from other islands adjacent to the New Zealand group. The maximum finding was 24 p.p.m.; the minimum, 1 p.p.m., and the average, 8.22 p.p.m.

Orr and Leitch (6) state that the amount of iodine found in soils ranges from 0.6 to 6.0 p.p.m., though amounts outside of these limits may be found. Soils from England and Scotland, analyzed at the Rowett Institute, contained from 2.4 to 8.0 p.p.m. of iodine; two soils from Kenya contained 3.0 and 3.5 p.p.m.; and one soil from the Falkland Islands contained 25.0 p.p.m. It is quite probable that the soil from Falkland Island, as well as that from

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

the New Zealand group, which contained a similar amount of iodine, has been enriched in iodine by the excreta and debris from the large flocks of sea birds which inhabit some of the islands and probably have done so for many years.

EXPERIMENTAL DATA

Figure 1 is an outline map of Kentucky showing the location and the approximate size of the six areas studied. Starting at the left these are numbered from 1 to 6.

The samples of soil used in this investigation were collected at different times in the past 20 years and have been kept in an air-dry condition, in closed glass jars, in the department of chemistry of the Kentucky Agricultural Experiment Station.

Method of determination

The combustion method used for the determination of iodine in the soils was that recently published by McHargue, Young, and Roy (5). In tables 1 to 6,

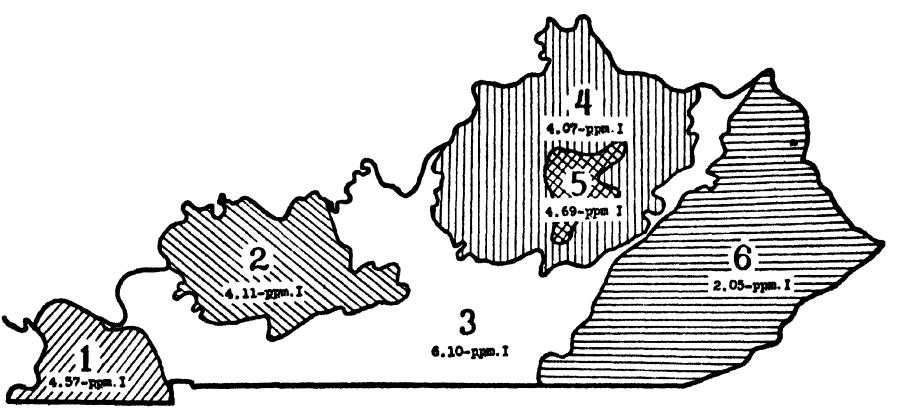

FIG. 1. OUTLINE MAP OF KENTUCKY SHOWING LOCATION AND APPROXIMATE SIZE OF AREAS IN WHICH IODINE CONTENT OF SOILS WAS DETERMINED

8, and 9, each result for iodine by combustion is the mean of two independent combustions of 10 to 100 gm. of soil. If the duplicate determinations were not consistent, a third was made and the mean of the two closest results taken. Agreement within about 8 per cent of the smaller figure was considered satisfactory.

The results under the heading "fusion" are single determinations, obtained by fusing 5 to 10 gm. of soil with potassium hydroxide in a large iron crucible, as in von Fellenberg's method, though the subsequent treatment was different from that described by von Fellenberg. The iodine was extracted from the salts with 95 per cent alcohol, liberated with sulfuric acid and sodium nitrite, and extracted with carbon disulfide, which was centrifuged and the solution compared by means of a micro-colorimeter, with a standard prepared in a similar way. All reagents were proved free from iodine before use.

Area 1—the Purchase

Area 1 is that part of the state which lies west of the Tennessee River, locally known as the "Purchase." It includes 8 counties, contains about 2,400 square miles and has a population of approximately 150,000. The soils are silt loams, underlaid by beds of gravel, sand, and clay of Quaternary, Tertiary, and Cretaceous age. The surface soil of a considerable part is loess. The average iodine content of the 42 samples, from 7 counties, is 4.57 p.p.m.;[2] the maximum, 6.93; and the minimum, 1.59, as determined by the combustion method. From these it appears that the soil of the Purchase area is fairly well supplied with iodine. Detailed results are contained in table 1.

TABLE 1

Iodine content as parts per million of air-dry soil from area 1—the purchase region

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Ballard	7	4.77	2.50–6.03	5.00
Carlisle	2	3.00	2.35–3.65	....
Fulton	8	4.74	2.60–6.35	5.90
Graves	6	5.34	4.65–6.93	5.43
Hickman	5	4.34	2.07–5.75	4.80
Marshall	9	4.08	1.59–5.70	5.90
McCracken	5	4.85	4.18–5.78	5.90
General average	42	4.45	1.59–6.93	5.49*

* Six determinations.

Area 2—the Western Coal Field

Area 2 contains approximately 4,850 square miles, in 11 counties, and has a population of about 265,700. The soils were derived largely from the disintegration of sandstones, shales, and clays, of Pennsylvania age, mostly in place. Some loess is present. Silt loams predominate. Sixty-nine samples, from 9 of the 11 counties in the area, were analyzed by the combustion method. The average iodine content of these is 4.11 p. p. m.; the maximum, 7.37, in a sample from Muhlenberg County; the minimum, 2.31, in one from Union County. The average for this area is slightly lower than that for the Purchase area. Table 2 contains the results in detail.

Area 3—Mississippian Area

The Mississippian area surrounds the Western Coal Field on the west, south, and east and extends to the western border of the Eastern Coal Field. The rocks are limestones, shales, and sandstones of Mississippian age. It contains

[2] To save space and for simplicity, the findings are stated as parts per million. The results by previous investigators usually have been stated in parts per billion.

approximately 11,667 square miles, in about 28 counties, and a population of approximately 455,100. The soils were derived largely from the disintegration

TABLE 2

Iodine content as parts per million of air-dry soil from area 2—the Western Coal Field

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Butler	4	4.31	4.10–4.76	3.40
Daviess	10	3.25	2.67–4.15	3.66
Henderson	10	5.20	4.30–6.87	6.50
Hopkins	7	4.00	3 61–4.41	4.58
McLean	4	3.39	2 91–3.80	3.28
Muhlenberg	10	4 90	3.92–7.37	6.10
Ohio	9	4.31	3 75–4 95	4.80
Union	6	3 07	2 31–3 75	2 50
Webster	9	3.77	3.35–4 21	3.60
General average	69	4 02	2.31–7 37	4 27*

* Nine determinations.

TABLE 3

Iodine content as parts per million of air-dry soil from area 3—the Mississippian

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Adair	8	5.80	4.00– 6.85	6.50
Barren	10	6.68	4.88– 8.75	7.30
Christian	12	6.80	3.98–16.70	4.57
Green	4	5.55	4.45– 6.15	6 50
Hart	9	6.48	5.17– 8.34	8 63
Hardin	5	5.43	4.68– 7.12	5.40
Larue	3	4.44	3.00– 5.80	4 80
Livingston	6	4.90	4.36– 5.80	5.50
Logan	9	9.88	3.60–16.95	3.80
Lyon	7	4.11	2.70– 4.98	3 00
Meade	6	4.26	3.45– 6.08	4 90
Metcalfe	6	4.38	3.50– 5.26	4.20
Taylor	2	6 64	6.30– 6.98	....
Todd	6	7.74	6.10– 8.88	9.60
Warren	7	5 16	2.53– 8 38	6 00
General average	100	6.10	2.53–16.95	5.76*

* Fourteen determinations.

of limestone rocks, in place, and they are generally silt and clay loams. One hundred samples, from 15 counties, were analyzed by the combustion method.

The average iodine content is 6.10 p.p.m.; the maximum, 16.95, in a sample from Logan County; the minimum, 2.53, in one from Warren County. This area gives a considerably larger general average for iodine than the others. The reason is probably because the red loam soil, derived from limestone, predominates in this area. The high results, 11, 12, and 16 p.p.m., indicate that a type of soil high in iodine occurs in this part of the state. The detailed results are stated in table 3.

TABLE 4

Iodine content as parts per million of air-dry soil from area 4—the Outer Bluegrass Region

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Bath	9	3.93	3.16–4.85	5.30
Boone	7	2.83	2.25–3.34	3.10
Bracken	4	4.53	4.20–4.85	5.29
Campbell	7	6.22	5.55–7.00	6.00
Grant	5	4.19	3.25–4.55	5.00
Harrison	11	3.99	3.10–4.90	3.50
Henry	9	2.71	1.10–4.85	4.80
Kenton	11	3.41	1.15–4.29	4.10
Lincoln	2	5.54	5.45–5.62	. . .
Madison	10	2.09	1.15–4.25	4.50 2.60
Montgomery	10	3.95	2.95–4.75	4.20
Nicholas	7	3.71	2.95–4.85	5.27
Owen	2	4.85	4.55–5.15	. . .
Pendleton	9	4.65	3.71–6.10	6.32
Robertson	2	4.10	3.75–4.45	. .
Shelby	13	3.59	1.95–6.00	4.01
Spencer	8	8.68	4.85–11.85	5.40
Washington	6	3.48	3.10–4.10	4.50
General average	132	4.24	1.10–11.85	4.50*

* Sixteen determinations.

Area 4—the Outer Bluegrass Region

Area 4 occupies the north central part of the state and contains approximately 6,722 square miles and a population of approximately 480,200. The soils were derived mainly from the disintegration, in place, of limestones and shales of Cincinnatian age. They are mostly silt and clay loams. One hundred and thirty-two samples, from 18 of the 30 counties in this area, were analyzed by the combustion method. The average iodine content is 4.07 p.p.m.; the maximum, 11.85, in a sample from Spencer County; the minimum, 1.10, in one from Henry County. The average for Spencer County, 8.68 p.p.m., is the second highest for any county in the state. The soils of this area are fairly well supplied with iodine but the element appears to be less abundant than in the soils of the Mississippian area. The findings are given in detail in table 4.

Area 5—the Inner Bluegrass Region

Area 5 occupies the south central part of the Bluegrass Region. It is also called the "Bluegrass Region proper," or the "Central Bluegrass Region." The area is characterized by the occurrence of highly phosphatic soil derived from limestone of the Lexington formation. Parts of some of the counties included here, however, do not belong to the Inner Bluegrass Region proper. Area 5 contains approximately 1,750 square miles and has a population of about 156,600. Most of the soils were formed in place, mainly from limestone strata, and silt loams and clay loams predominate. Sixty-four samples, from the 7 counties, were analyzed by the combustion method. The average iodine content is 4.69 p.p.m.; the maximum, 8.25, in a sample from Mercer County; and the minimum, 2.40, in one from Bourbon County. The average is slightly

TABLE 5

Iodine content as parts per million of air-dry soil from area 5—the Inner Bluegrass Region

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Bourbon	9	3.80	2.40–5.25	4.40
Clark	9	3.18	2 65–3 60	3.50
Fayette	14	5.45	3.65–7.29	6.20 4.80
Jessamine	7	4.00	3.16–4 46	3.50
Mercer	9	5.45	3.14–8.25	5.63
Scott	6	4.08	3.49–4.59	3.90
Woodford	10	3.77	2.05–4.50	4.10
General average	64	4.35	2.05–8.25	4.50*

* Eight determinations.

larger than that for the soils of the outer bluegrass area. Because much of the soil of the central area was derived from the disintegration of phosphatic limestone, higher results for iodine were anticipated.

Area 6—the Eastern Coal Field

Area 6 forms the eastern part of the state and contains about 8,760 square miles, of hilly to mountainous topography. The tallest peaks exceed 4,000 feet above sea level. The population is approximately 638,500. The soils are sandy loams to silt loams, in the main, derived from the disintegration of sandstones and shales of Pennsylvanian age.

Only 14 samples, from 6 counties, were available for iodine determination. The maximum iodine content found by the combustion method is 3.08 p.p.m., in a sample from Pike County; the minimum, 0.81, in one from McCreary

County. The average for the 14 samples is 2.05 p.p.m., which is about half the average of the soils in the Western Coal Field area. It is therefore evident that the mountainous area contains a smaller amount of iodine in the soil than any of the other areas. Although the number of samples analyzed for iodine was smaller than in any other area of near the same size, the samples were taken in the proper manner and it is believed that they give a fair representation of the iodine content of the soils of this part of the state.

TABLE 6

Iodine content as parts per million of the air-dry soil of area 6—the Eastern Coal Field

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Lawrence	3	2.14	1.40–2.53	2 46
Leslie	1	2 23	....	1.95
Magoffin	1	1 58	. .... .	1 75
Martin	2	2 15	1.87–2 42	2.50
McCreary	1	0 81	.....	....
Pike	6	2.24	1.50–3.08	2.00
General average	14	2.05	1 40–3.08	2 13*

* Five determinations.

TABLE 7

Summary of the iodine content of soil from the six areas, stated as parts per million of air-dried soil

AREA	Number of counties represented	Number of samples analyzed	IODINE CONTENT		
			Maximum	Minimum	Average
			p.p.m.	*p.p.m.*	*p.p.m.*
Area 1—Purchase	7	42	6 93	1.59	4.57
Area 2—Western Coal Field	9	69	7.37	2.31	4.11
Area 3—Mississippian	15	100	16 95	2.53	6.10
Area 4—Outer Bluegrass Region	18	132	11.85	1.10	4.07
Area 5—Inner Bluegrass Region	7	64	8 25	2.40	4.35
Area 6—Eastern Coal Field	6	14	3.08	0.81	2.05
All areas	62	421	16 95	0 81	4.59

Tables 8 and 9 contain the results for iodine determined by the combustion method in samples of soil taken to represent the different soil horizons of the Maury and Mercer silt loam types, respectively. The Maury soil was derived from the disintegration of phosphatic limestones and the phosphorus content increases more or less uniformly from 0.33 per cent in the surface soil to about

10.5 per cent in the lowest stratum. The surface layer contains the largest amount of iodine and the subsequent layers vary irregularly in iodine as the depth increases. The average for the whole profile is 5.16 p.p.m.

TABLE 8

Iodine content of Fayette County soil, Maury type, taken at different depths, air-dry

NUMBER	SOIL HORIZON	DEPTH	CHARACTER	IODINE
		inches		*p.p.m.*
422	A 1	0–8	Brown silt loam	8.95
423	A 2	8–17	Reddish brown silt loam	6.60
424	B 1	17–34	Reddish brown silty clay loam	4.40
425	B 2	34–66	Silty clay loam	4.00
426	C 1	66–72	Dark chocolate-brown	7.70
427	C 2	72–86	Very dark chocloate-brown. Semi-weathered	5.80
428	C 3	86–104	Dark brown silty clay	4.40
429	C 4a	104–x	Dark chocolate-brown silty clay	4 05
430	C 4b	x–130	Like C 4a	4 00
431	C 5	130–144	Blackish, gritty material	7.00
		Rock		
Average*				5.16

* Computed on the assumption that the weight of soil in each division is proportional to its thickness.

TABLE 9

Iodine content of Fayette county soil, Mercer silt loam type, taken at different depths, air-dry

NUMBER	SOIL HORIZON	DEPTH	CHARACTER	IODINE
		inches		*p.p.m.*
432	A_1	0–7	Dull brown silt loam	6.00
433	A_2	7–20	Rusty brown silt loam	4.70
434	A_3	20–36	Granular silt loam	5.00
435	B_1	36–54	Brown and rusty yellow clay	6 70
436	B_2	54–76	Bluish and pale yellow clay	9 70
437	C_1	76–86	Blue, gray rusty brown clay	4.70
438	C_2	86–96	Blue, yellow and brown clay	4.00
439	C_3	96–116	Yellow clay (alkaline)	6.30
Average*				6.29

* Computed on the assumption that the weight of soil in each division is proportional to its thickness.

Samples of limestone from a fresh excavation near the place where the samples of soil for the Maury profile were taken, were analyzed for iodine. A sample from near the top of the underlying rock contained 0.25 p.p.m. of iodine. A fossiliferous layer about 3 feet lower contained 2.0 p.p.m. of iodine. There-

fore, iodine occurs in greater concentration in soil than in the underlying limestone from which the soil was derived.

Plates of phosphate rock taken from soil immediately above the underlying limestone, were analyzed for phosphorus and iodine. The sample contained 75.0 per cent of tricalcium phosphate and 20 p.p.m. of iodine. The high result for iodine in the phosphate indicates that this material contains an iodine mineral, possibly similar in composition to appatite, which mineral contains fluorine and is associated with phosphate rock.

The Mercer silt loam soil was derived from limestone low in phosphorus. The maximum phosphorus content of the soil of this profile is approximately 0.3 per cent and the minimum, 0.1 per cent. The maximum iodine content of the Mercer silt loam, 9.7 p.p.m., occurred below the middle of the profile, and the minimum, 4 p.p.m., still lower. The average for the profile was 6.29 p.p.m. The results for iodine in the two soil profiles show that there is no close correlation between iodine content and phosphorus content in these two soils.

DISCUSSION

Fusion analyses of 58 samples of soil from as many counties averaged 4.70 parts of iodine per million of dry soil, whereas combustion analyses of the same samples averaged 4.44 p.p.m. The result is confirmatory of the findings by the combustion method.

It is to be observed that the soils in area 3 contain the largest quantity of iodine and those in area 6 the smallest. However, an inspection of the individual results in tables 1 to 6 shows that a few low results are contained in each, which is an indication that each area contains soils that are low and possibly deficient in iodine, from the standpoint of agriculture. On the other hand, there are a few soils in the state that are exceptionally rich in iodine. How extensive the high and low iodine areas are remains to be ascertained.

The soils of the Purchase Region are unique inasmuch as they were formed in a different way from those of the other five areas. Though largely derived from powdered rock material which was carried to its present location by streams of fresh water and deposited in an ancient embayment, they contain more loessal material supposed to have been brought by the wind from a westerly direction, than those of any other area. The water-borne débris has undergone considerable weathering and probably has lost a part of the iodine which it contained at the time it was eroded from the rock. The fact that the soils of this area rank third among those of the other geological areas of the state in iodine content is of considerable interest and suggests that possibly the soil material was eroded from rocks containing considerable iodine a part of which they have retained until the present time.

The soils of the Western and Eastern Coal Field areas were derived mainly from the disintegration of sandstone and shale strata in place. The soils of the Eastern Coal Field contain about half as much iodine as those in the Western Coal Field. Apparently the soils of mountainous regions elsewhere

have been found to contain less iodine than those at lower altitudes. The soils of the Eastern Coal Field seem to follow this general rule. Because the soils of the Eastern Coal Field are low in iodine we would expect a greater incidence of goiter in this area than elsewhere in the state, as well as of those affections of livestock which attend iodine deficiency.

The soils of the other three areas have a general relationship in that they were derived mainly from the disintegration of limestone strata. Soils derived from limestone received their iodine from marine sources rather than from particles of undecomposed rock and probably have retained it by adsorption. The sea has long been regarded as the principal source of iodine. However, Orr and Leitch (6) point out that the iodine content of soils ranges from 0.6 to 6.0 p.p.m. and that the earth's crust, and not the sea, is the principal storehouse of iodine.

It seems reasonable to suppose that as particles of calcium and magnesium carbonate were precipitated in sea water they adsorbed and held tenaceously, molecules of some iodine compound derived from the débris from marine organisms and plants which was rich in iodine; accordingly, limestone strata contain more or less iodine, and the amount in Kentucky limestones appears to vary with the purity of the strata, the crystalline deposits containing the smallest quantity. Orr and Leitch (6) present data which show that fossiliferous limestones contain nearly twice as much iodine as non-fossiliferous deposits. Soils derived from the former should contain a larger quantity of iodine. However, they state that a wide range of variation in iodine content exists within each formation and that no sharp distinction can be drawn between the amount of iodine contained in different strata. Hence rocks belonging to the same formation from different districts may differ widely in their iodine content.

As the limestone strata weather from the top surface, beneath the soil, small quantities of iodine compounds may go into solution and may be either carried away in drainage waters or left in the débris, adsorbed by particles of clay and iron oxide. An iodine compound that resists weathering, such as that in the phosphate plates, would remain. Consequently, the disintegration of limestone should give rise to soils which contain considerable quantities of iodine. This may account for the high iodine content of some of the soil samples from the Mississippian, Outer Bluegrass, and Lexington areas in Kentucky. Then, too, the red clay soils which predominate in the Mississippian area and the other areas of limestone origin probably have peculiar adsorptive properties due to the hydrous ferric oxide which they contain in considerable amount.

SUMMARY

Four hundred and thirty-nine samples of soil from the six principal geological areas in Kentucky were analyzed for iodine. The largest quantities were found to be associated with soils derived from limestone strata. The smallest quantity of iodine occurred in the soils derived from sandstone strata, in the Eastern Coal Field area. It therefore appears that the foods and natural waters produced in some parts of the Eastern Coal Field may be deficient in

iodine to the extent that endemic goiter is more likely to be prevalent in this part of the state than in any other part. The soils of the Western Coal Field area contain nearly twice as much iodine as those of the Eastern Coal Field. The iodine content of the Maury (phosphatic) and the Mercer (non-phosphatic) soil profiles in Fayette County show considerable variation at different depths. There was no correlation between the phosphorus and iodine content in the two profiles. Soils derived from limestone rocks contain considerably more iodine than the unaltered rock.

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STUDIES ON READILY SOLUBLE PHOSPHATE IN SOILS: I. EXTRACTION OF READILY SOLUBLE PHOSPHATE FROM SOILS BY MEANS OF DILUTE ACID POTASSIUM SULFATE ($KHSO_4$)[1]

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Many different methods have been proposed for the extraction of phosphate from soils by means of dilute acids in order to get an estimate of their fertility in respect to phosphate. One group of these methods is based upon the different hypothesis dealing with the plant's absorption of nutrients from the soil, and in one way or another the scope has been to approximate this absorption. Another group of such methods is based upon chemical considerations, and it is often assumed that the compounds in the soil are of the same constitution and nature as the common known synthetic phosphate compounds. It seems logical to assume that the usefulness of any chemical fertility method is mostly dependent upon how the results can be interpreted. If, by an extraction method, results can be obtained which are on a uniform basis in respect to the most important factors determining the solubility of the nutrients, for the case here—of phosphate, it is to be expected that the results will be interpretable. The researches presented in this paper are part of a series of researches on phosphates started in July, 1931.

REVIEW OF LITERATURE

Comprehensive investigations of Hoagland and his co-workers (16, 18, 17, 43) on the plant's intake of nutrients from the medium of growth have fully shown that the laws governing these processes are much more complicated than is usually assumed by the chemists who have proposed methods on such a basis.

It is an important fact, however, that the solubility of the compounds of N, P, and K in the medium of growth and the amounts taken up by the plants are rather closely correlated. In general, the more easily soluble N, P, and K there are in the medium of growth, the higher is the total content of these nutrients in the crop (1, 19).

That the plants, to some extent, can very likely get their supply of phosphate by direct action on the solid soil phase has been pointed out in a very logical way by several investigators (5, 24, 28, 30). Thus, Comber emphasizes that the soil and the plant form one sys-

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tem as the root hairs and the soil particles are cemented together. This offers the possibility that the cell sap might have dissolving action directly on the soil particles, and thus both inorganic and organic compounds in the cell sap may account for the dissolving action of the plant roots on the soil compounds. The fact that the phosphates common in soils (under the acidity conditions prevailing in many soils) may be entirely precipitated from the soil solution (11) supports the assumption that the plant roots very likely have a directly dissolving action on the solid soil phase. For the case of calcareous soils, solvents other than carbon dioxide must be of major importance in dissolving the soil phosphates (33). McGeorge and Breazeale (23) have shown that black alkali soils which contain sufficient replaceable Na to yield free hydroxyl ions on hydrolysis, contain readily soluble phosphate in large amounts, but the high alkalinity checks its absorption by the plants. Some investigations also indicate that there may be more water-soluble phosphate in some soils than the plants assimilate (7, 39).

From the literature reviewed here, it becomes apparent, that an estimation of the amount of *available* phosphate in a soil offers considerable difficulties.

Rindell (29) long ago called attention to the importance of a more detailed control in chemical studies on products as soils and the like. The same author recognized such problems as re-precipitation of dissolved phosphate in phosphate extractions, acidity control of the extracts, and the importance of the ratio of the solid mass to the solvent.

Very comprehensive studies on the solubility of soil phosphates by von Wrangell and her co-workers (39, 40, 41, 42) have revealed that the soil phosphates must be largely of a very complex nature, and their solubility is far less than that of the synthetic tertiary Ca- and Mg-phosphates and the synthetic phosphates of Al and Fe.[4] Wrangell also points out that the laws of diffusion do not govern the dissolution rate of these phosphates, but the dissolution rate is governed by the rate of the decomposition of the surface of the particles which contain the phosphate. Gregoire (13) concludes that the water-soluble soil phosphate mainly originates from colloidal compounds.

Hibbard (15), on the basis of a comprehensive study of the solubility of soil phosphates in different solvents, has come to the conclusion that the amount of phosphate dissolved in acid extract increases when the hydrogen-ion concentration and the relative volume of the solution increases. By extracting soils with a large number of neutral salt solutions, appreciable amounts of phosphate only were dissolved by using ammonium salts of oxalic and citric acid.

The effectiveness of acid solvents is obviously due to the small volume of the hydrogen ion which, because of this, is able to enter into very fine openings, and it can also come into closer proximity with negatively charged particles and be held more firmly than any other positive ion (26). The capillary and porous structure of the soil colloids is, no doubt, very important for their disintegration (14, 25, 35).

The solubility of the phosphates commonly existing in soils is mainly determined by the acidity of solution (2, 8, 9, 11, 35). In the figures obtained in these investigations, some minor disagreements exist, due to the different experimental methods used. The investigations reveal, however, that the different phosphates have a distinctive range of minimum solubility. When the bases used in soils are in excess, the phosphates can be almost completely precipitated from their solutions. The following figures from Gaarder's detailed investigations (11) give an illustrative picture of the behavior of some of the most important phosphates found in soils.

	pH range of minimum solubility
Excess of Fe-hydroxide	3 –4
Excess of Al-hydroxide	5 –6
Excess of Al-hydroxide + Ca	5 –6.3
Excess of Al-hydroxide + Mg	4.6–6.5

[4] Fe means in this paper Fe^{III}.

In these pH ranges, the phosphates are practically completely precipitated from their solutions.

The studies on the precipitation of phosphates explain the fact that re-precipitation of dissolved phosphate may take place during extraction of phosphate from soils if the soil remains in contact with the extract for a long time and the acidity of the solvent is below about a pH of 3. That such re-precipitation actually takes place in extraction of soil phosphate with dilute acids becomes apparent from the studies on the action of dilute acids on phosphorus compounds in soils by Russell and Prescott (32). Re precipitation was most marked for dilute HNO_3, less for HCl, and least for H_2SO_4. This phenomenon was recognized by the fact that short-time extractions often yielded more phosphate to the solutions than did long-time extractions, and it was later proved by Comber (6).[5]

METHODS OF EXPERIMENTATION

To determine the small amounts of phosphate in soil extracts, the Deniges colorimetric method, as worked out by Truog and Meyer (37) and somewhat modified by Chapman (4), has been used.

The extraction method of Truog (36) for extracting "readily available" phosphate from soils has been used in some of the experiments. By this extraction method a solvent of 0.002 *N* H_2SO_4 buffered with $(NH_4)_2SO_4$ to a pH of 3 is used. Two grams of soil is shaken with 400 cc. solvent for half an hour.

If not otherwise stated, air-dried soil passed through a 20-mesh sieve is used. The pH determinations in all extracts are made by means of the quinhydrone electrode. For soil suspensions, both the quinhydrone electrode and the colorimetric methods have been used. All extractions were made at a temperature of 20 to 22°C.

EXPERIMENTAL DATA

The purpose of the experimental work carried out has been to find a suitable inorganic acid solvent for extraction of the more readily soluble phosphate from soils, and to work out a technic for such extractions.

Experiments with a series of different solvents revealed that a solvent of dilute acid potassium sulfate ($KHSO_4$) possesses distinct qualities for the purpose of extraction of readily soluble soil phosphate.[6] This solvent is, in concentrations of 0.01 *M* and upwards, appreciably buffered as a result of the partial dissociation of the HSO_4-ion.

The salt measured was *Potassium Bisulfate Merck C.P. Crystals* ($KHSO_4$). As the salt is deliquescent, itwas dried at 33–35°C. for one week. The pH values were made by means of a quinhydrone electrode using a saturated

[5] Comber (6) showed that the velocity of dissolution of soil phosphate was much more rapid than was the velocity of solution of Fe- and Al-hydroxides, which cause re-precipitation. Extraction of a soil with 0.05 *N* HNO_3 gave the following result:

	P_2O_5	$Fe_2O_3 + Al_2O_3$
After 10 minutes, mgm. per 100 cc.	7.39	63 0
After 24 hours, mgm. per 100 cc.	4.56	221.0

[6] In recent investigations of Mitchell (27) $KHSO_4$ has also been found very suitable for extraction of soil phosphate.

calomel cell and a Leeds and Northrup pH indicator. Stocks of $KHSO_4$ from different sources have varied somewhat in acidity at the same concentrations.

The soil extracts with $KHSO_4$ were easily filtered because of the flocculating effect of this solvent. Also, organic matter, iron, and silica were not dissolved in sufficient amounts to interfere with the colorimetric determinations.

$$x\ KHSO_4 \rightleftharpoons x\ K^+ + x\ HSO_4^-,$$
$$x\ HSO_4^- \rightleftharpoons x\ H^+ + (x - y)\ HSO_4^-,\ (x > y)$$

TABLE 1

The hydrogen-ion concentration of $KHSO_4$-solutions of different concentrations

$KHSO_4$ IN 1,000 CC.	MOLAR CONCENTRATION	pH OF THE SOLUTION
gm.		
13.617	0.100	1.40
2.723	0.020	1.91
2.383	0.018	1.95
2.092	0 015	2.00
1.362	0 010	2.14
0.800	0.006	2.32
0.650	0.005	2.40
0.500	0.004	2.50
0.250	0 002	2.74
0.136	0.001	3.06
0.0136	0 0001	4.08

TABLE 2

The influence of varying amounts of soil to 200 cc. 0.01 M $KHSO_4$ upon the amounts of phosphate dissolved from two soils

WELLAND SOIL (SURFACE; pH 4.61)				MARKDALE SOIL (SURFACE; pH 7.63)			
Soil used	P in extract	P in 1,000 gm. soil	pH of extract	Soil used	P in extract	P in 1,000 gm. soil	pH of extract
gm.	*mgm.*	*mgm.*		*gm.*	*mgm.*	*mgm.*	
2	0.210	105	2 31	2	0.044	22	2.46
4	0.325	81	2.32	4	0.055	14	2.56
10	0.725	73	2.49	10	0.089	9	3 09
50	0.170	3	3.28	50	0.029	0.6	5.48

Various amounts of two soils were shaken 5 minutes with 200 cc. 0.01 *M* $KHSO_4$ and filtered rapidly at once (suction) through a Buchner funnel with an inside diameter of 9½ cm. (# 42 acid-washed Whatman filter paper).[7] The pH of the solvent was 2.30 (the disagreement in this respect with the results in table 2 is due to the fact that the salt used was of another preparation).

[7] In all experiments presented here, this size of funnel is used in order that the filtering can be as rapid as possible. Also, Whatman # 42 acid-washed filter paper only is used. (The filter papers were always tested before use.)

It is significant that with the first three amounts of soil, the pH values of the solvent after the extraction have not gone above 3.09[8] and the amounts of phosphate extracted are proportional with the amounts of soil used. By using 50 gm. of soil to 200 cc. of this solvent, the acidity of the solvent is

TABLE 3

The influence of removal of dissolved organic matter and silica upon the phosphate content in 1:200 0.01 M $KHSO_4$-extracts of two soils

SOIL	TIME OF SHAKING	P IN 1,000 GM. SOIL	
		Organic matter and silica removed	Organic matter and silica *not* removed
	minutes	*mgm.*	*mgm.*
Markdale surface soil	15	27	22
	30	33	27
Markdale subsoil*	15	29	20
	30	32	23
Welland No. 2 surface soil	15	149	129
	30	204	174

* pH 7.51.

TABLE 4

*Solubility of soil phosphate in two subsoils at different acidities**

SOIL	pH OF SOLVENT BEFORE EXTRACTION	pH OF SOLVENT AFTER EXTRACTION	P DISSOLVED PER 1,000 GM. SOIL
			mgm.
Markdale subsoil (pH 7.51)	2.18	2 22	23
	2 00	2.04	30
	1 80	1 81	32
	1.44	1.46	51
Welland subsoil (pH 5.22)	2 18	2.18	22
	2 00	2.04	25
	1.80	1.84	32
	1.44	1 45	35

* Experiments with the Markdale soil with extractions with increasing amounts of HCl and HNO_3 showed the same trend.

decreased to a range where re-precipitation of dissolved phosphate takes place, and the phosphate content of the extracts is consequently very low.

In the evaporated aliquots organic matter was destroyed by ignition, after

[8] It is also important to note that the pH of 3.09 is obtained in extraction of the Markdale soil, which is a highly calcareous soil.

TABLE 5

The influence of time of shaking upon the amounts of phosphate extracted from soils in 1:200 extracts with $KHSO_4$ at pH 2.00

SOIL	TREATMENT IN THE FIELD	TIME OF SHAKING	P IN 1,000 GM. SOIL	pH AFTER EXTRACTION
		minutes	*mgm.*	
Markdale surface	Summer fallow*	5	23	2.01
		15	26	2.02
		30	29	2.02
Markdale subsoil		5	24	2.05
		15	28	2.07
		30	31	2.10
Welland permanent fertility plots:				
No. 1 surface	400 lbs. acid phosphate per acre	5	39	2.00
		15	47	2.00
No. 2 surface	1,000 lbs. rock phosphate + 500 lbs. S per acre	5	180	2.00
		15	186	1.99
		30	224	2.00
subsoil	*	5	23	2.05
		15	25	2.03
		30	25	2.05
No. 3 surface	2 tons lime per acre	5	27	2.02
		15	27	2.03
No. 4 surface	2 tons lime + 400 lbs. acid phosphate per acre	5	38	2.00
		15	48	2 01
No. 5 surface	2 tons lime + 1,000 lbs. rock phosphate per acre	5	248	2.01
		15	260	2.00
No. 6 surface	2 tons lime + 600 lbs. basic slag per acre	5	69	2.03
		15	69	2.02
No. 7 surface	2 tons lime + 100 lbs. sulfate of ammonia + 400 lbs. acid phosphate + 100 lbs. muriate of potash per acre	5	41	2.02
		15	45	2.02
No. 11 surface	No treatment	5	24	2.01
		15	33	2.01

* The aforementioned Markdale and Welland soils.

evaporation with $Mg(NO_3)_2$, and silica was removed by centrifugation after acid dehydration.

To determine the influence of the acidity of the solvent upon the solubility of difficultly soluble soil phosphate, 2-gm. portions of each of the subsoils from Markdale and Welland were shaken 15 minutes with 400 cc. of $KHSO_4$ solutions of different acidities.

TABLE 6

Readily soluble phosphates extracted from phosphate treated soil samples

SOIL IN 200 CC. KH_2PO_4 SOLUTION	P REMOVED BY SOIL FROM KH_2PO_4 SOLUTION	AMOUNT OF P EXTRACTED FROM SOIL BY $KHSO_4$ METHOD*		INCREASE OF DISSOLVED P DUE TO THE P REMOVED FROM KH_2PO_4 SOLUTION	PER CENT OF THE P REMOVED BY SOIL RECOVERED IN $KHSO_4$ EXTRACT
		Phosphate treated soil	Untreated soil		
gm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Welland soil:					
Surface soil					
100	22.6	21 0	18.0	3.0	13
50	20.3	14.7	9.0	5.7	28
10	7.0	5.4	1.8	3.6	51
Subsoil					
100	22 4	10 7	3.0	7.7	34
50	19 6	11 0	1.5	9 5	48
10	7.7	3 4	0.3	3.1	40
Markdale soil:					
Surface soil					
100	22.6	10.7	2.3	8.4	37
50	19 3	10 8	1.2	9 6	50
10	10.4	2.8	0.2	2.6	25
Subsoil					
100	21.4	10.5	2 4	8.1	38
50	15.5	9.3	1.2	8 1	54
10	9.2	3.0	0 2	2.8	30

* Comparisons between the Truog and the $KHSO_4$ methods are given in tables 7, 8, and 9.

Then the $KHSO_4$ solution at a pH of 2.00 was chosen for a study of the influence of time upon the rate of dissolution of soil phosphate from soils well known in so far as their phosphate treatment and response to phosphatic fertilizers was concerned. Two grams of soil was shaken 5 minutes with 400 cc. of $KHSO_4$ and filtered at once. The $KHSO_4$ stock used for the measurements reported in table 1 was used here and in all the following experiments.

As the amounts of the difficultly soluble soil phosphates dissolved do not increase appreciably as a result of prolonged shaking, 5 minutes of shaking and 2 gm. of soil to 400 cc. $KHSO_4$ at a pH of 2.00 were chosen for further experi-

TABLE 7

Readily soluble phosphate in samples of surface and subsoils from Welland fertility plots, as determined by the $KHSO_4$ and the Truog methods

PLOT NUMBER	pH OF SUSPENSION (1:1)	$KHSO_4$ METHOD		TRUOG METHOD	
		P per 1,000 gm. soil	pH of extracts	P per 1,000 gm. soil	pH of extracts
		mgm.		*mgm.*	
Surface soils					
1	5.67	39	2.02	22	3.15
2	4.61	180	2.00	92	3.12
3	6.11	24	2.02	16	3.05
4	5.58	38	2.00	26	3.08
5	6.30	248	2.01	207	3.10
6	6.92	69	2.03	..	....
7	6.15	41	2.02	..	....
10*	5.54	32	2.01	..	....
11	5.25	24	2.01	..	....
Subsoils					
1	6.00	116	2.05	76	3.15
2	5.22	23	2.02	11	3.01
3	5.83	59	2.02	41	3.03
4	5.46	57	2.02	43	3.07
5	5.63	80	2.02	52	3.20
6	6.06	110	2.03	..	....
7	5.53	47	2.01	..	....
10*	5.25	12	2.03	..	....
11	5.11	9	2.03	..	....

* Plot 10 has received 2 tons lime + 100 pounds sulfate of ammonia + 400 pounds acid phosphate per acre. For explanation of the treatment of the other plots, see table 5.

TABLE 8

Readily soluble phosphate in the profile of the Guelph sandy loam as determined by the $KHSO_4$ method and the Truog method

HORIZON	pH*	TOTAL CONTENT OF P PER 1,000 GM. SOIL	$KHSO_4$ METHOD		TRUOG METHOD	
			P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil	pH of extract
		mgm.	*mgm.*		*mgm.*	
A_1	7.94	1,267	38	2.03	24	3.24
A_2	7.68	1,049	18	2.04	11	3.15
A_3	7.68	1,092	47	2.02	25	3.10
B_1	7.66	1,136	69	2.02	38	3.42
B_2	8.27	1,223	122	2.06	39	5.15
C_2	8.01	786	56	2.33	..	....
..	....	.....	73†	2.02†	7	6.54

* Analysis after Ruhnke (31).

† By adjustment of the solvent with 0.700 gm. $KHSO_4$.

mentation. In the following for convenience this extraction is called the "$KHSO_4$-method."

In order to get an idea as to the dissolving capacity under these experimental conditions, the method was tried on samples from a fixation experiment with the Welland and Markdale soils. To 200 cc. of a KH_2PO_4-solution containing

TABLE 9

Readily soluble phosphate in a fertile garden soil as determined by the $KHSO_4$ and the Truog methods*

	P per 1,000 gm. soil	pH of extracts
	mgm.	
Truog method	290	5.27
$KHSO_4$ method	450	2.05

* Total phosphorus = 1,200 mgm. P in 1,000 gm. soil. pH 8.18.

TABLE 10

Soil phosphate dissolved in $KHSO_4$ solvents at the pH values of 3.00 and 3.80

Number of plot	$KHSO_4$ at a pH of 3.00		$KHSO_4$ at a pH of 3.80	
	P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil	pH of extract
	mgm.		*mgm.*	
Surface soils				
1	12.5	3.20	9	4.45
2	43	3.12	16.5	4 10
3	8.5	3 27	6	5.07
4	14 5	3.20	8.5	4 91
5	88	3.22	16	4.58
Subsoils				
1	41	3.17	14.5	4.42
2	3.5	3.13	2	4.47
3	22	3.16	9	4.58
4	20	3.16	8.5	4.50
5	23	3 17	10	4.58
Garden soil surface	206	4 98	74	6.01

25 mgm. P 100, 50, and 10 gm. of soil were added and left standing, with frequent shaking, for 17 hours, following which the phosphate content in the solution was determined. Then the soil was sucked as dry as possible on a large Buchner funnel, air-dried, and extracted by the $KHSO_4$-method as described. Samples of the same soils without phosphate treatment were also extracted.

$KHSO_4$ solvents of other concentrations have been used with five samples

from the Welland plots and the garden soil (2 gm. soil to 400 cc. $KHSO_4$, 5 minutes of shaking, see table 10, and fig. 1).

Surface soils 1, 2, and 3 from Welland were shaken 5 minutes with CO_2-free distilled water (soil:solvent = 1:100). No dissolved phosphate could be

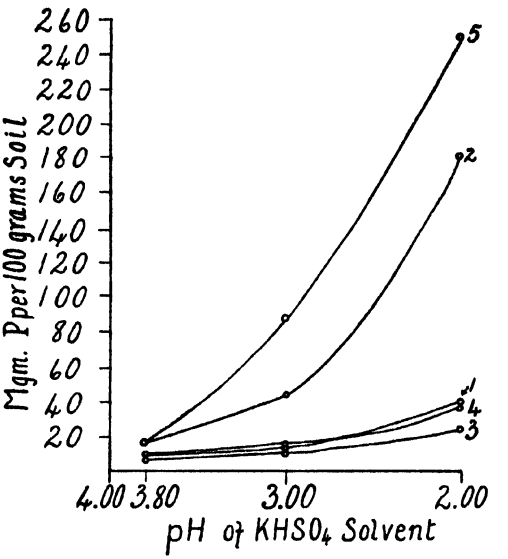

FIG. 1. SOIL PHOSPHATE DISSOLVED IN $KHSO_4$ SOLVENTS OF DIFFERENT ACIDITIES
Samples from Welland Plots

detected. By repeating water extraction in the same way after having dehydrated the soils 36 hours at 105°C. the following results were obtained:

SOIL NUMBER	P PER 1,000 GM. SOIL	pH OF EXTRACT
	mgm.	
1	8	5.41
2	9	5.49
3	Negligible	5 59

The garden soil (see table 9), in which the amount of readily soluble phosphate is rather high, was leached with several solvents, and the phosphate content in the subsequent leachates determined. Ten grams of the fresh soil was spread in a thin layer on a Buchner funnel (9.5 cm. in diameter, Whatman # 42, acid-washed filter paper), and 100-cc. portions of the different solvents were poured on in portions of 25 cc. (see table 11 and fig. 2).

The leachings with the $KHSO_4$ (pH 2.00) were continued until all of the more readily soluble phosphate was removed (see table 12).

Figure 3 shows that 10 leachings with $KHSO_4$ (at a pH of 2.00) have re-

TABLE 11

The influence of the acidity of the solvent in dissolving soil phosphate by leaching of a fresh fertile garden soil

NUMBER OF LEACHATE	DISTILLED WATER (FREE FROM CO_2)		0.002 *N* H_2SO_4 BUFFERED TO pH 3 00 (SOLVENT TO TRUOG METHOD)		$KHSO_4$ AT pH 2.00 (SOLVENT TO $KHSO_4$ METHOD)		0.1 *N* KCl + HCl; pH 1.50	
	P in 100 cc. fresh leachate	pH of leachate	P in 100 cc. leachate	pH of leachate	P in 100 cc. leachate	pH of leachate	P in 100 cc leachate	pH of leachate
	mgm.		*mgm.*		*mgm.*		*mgm.*	
1	0 132	6.78	0 250	6 58	0 800	2 81	1.150	1 97
2	0 059	6 95	0 225	7 06	0 633	2 51	0 800	1 79
3	0.055	6 85	0.193	6 99	0 500	2 37	0 562	1.66
4	0 048	....	0 178	7 05	0 450	2.37	0 465	1 67
5	0 046	6 90	0 173	7 15	0 400	2.31	0 375	1 64
Total amount of P leached out in 5 leachings, mgm. P.	0 340		1 019		2 783		3.352	

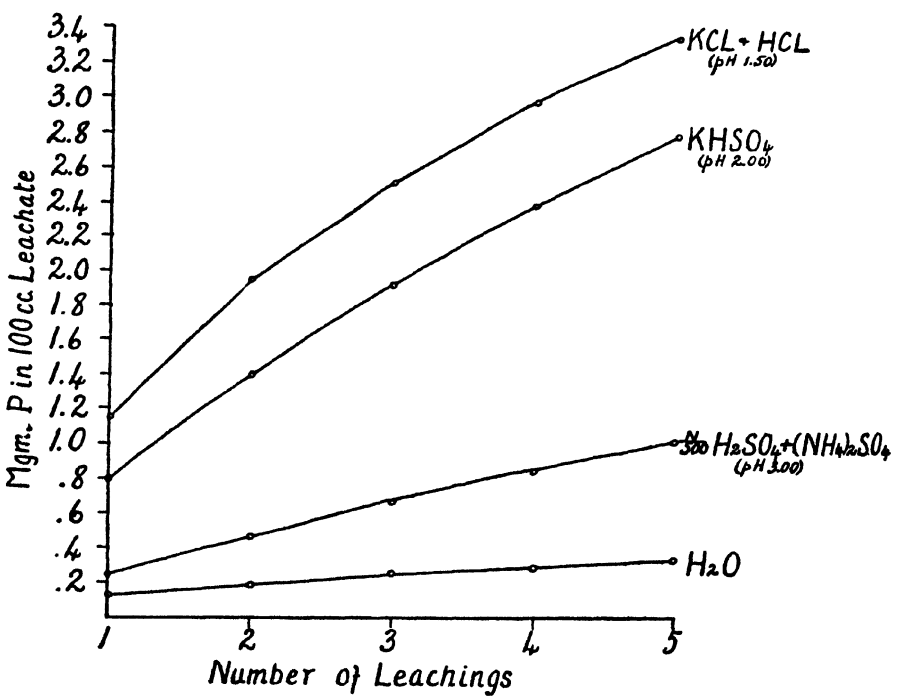

FIG. 2. THE INFLUENCE OF THE ACIDITY OF THE SOLVENT IN DISSOLVING SOIL PHOSPHATE BY LEACHING A FRESH FERTILE GARDEN SOIL

TABLE 12

Leaching of a fresh fertile garden soil with $KHSO_4$ at a pH of 2.00*

NUMBER OF LEACHING	P IN 100 CC. OF LEACHATE	pH OF EXTRACTS	NUMBER OF LEACHING	P IN 100 CC. OF LEACHATE	pH OF EXTRACTS
	mgm.			*mgm.*	
1	.800	2.81	17	....	....
2	.633	2.51	18	.130	2.30
3	.500	2.37	19	....	....
4	.450	2.37	20	.092	2.25
5	.400	2.31	21	....	....
6	.270	2.37†	22	.090	2.26
7	....	....	23	....	....
8	.240	2.30	24	.078	2.26
9	....	....	25	....	....
10	.180	2.22	26	.094	2.23
11	....	....	27	....	....
12	.129	2.19	28	.080	2.21
13	....	....	29	....	....
14	.108	2.20	30	.063	2.22
15	....	....†	31	....	....
16	.130	2.26	32	.058	2.22

* Fresh soil — per cent moisture = 16.32 (dried 24 hours at 105°C.). Air-dried soil — per cent moisture = 2.51.

† Interruption.

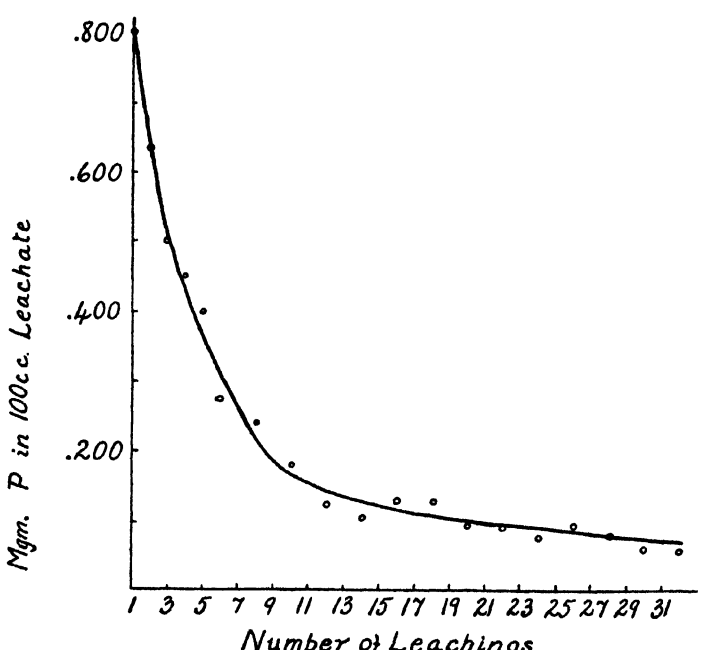

FIG. 3. CONTINUED LEACHING OF A FRESH FERTILE GARDEN SOIL WITH $KHSO_4$ AT A pH VALUE OF 2.00

moved about all of the readily soluble soil phosphate as extracted at this acidity. Calculated on air-dry basis, the leachates from the 10 leachings contained 457 mgm. of P per 1,000 gm. of soil. Five minutes of shaking the air-dry soil with the same solvent removed 450 mgm. of P per 1,000 gm. of soil (table 9). Thus, it is seen that 5 minutes of shaking removes practically all of the more readily soluble phosphate.

The $KHSO_4$ solvent, pH 2.00, has also been tried on phosphatic materials. The amounts of P dissolved from such materials are far greater than the amounts found in any soil. In table 13 are given the results from extractions of a rock phosphate, which has been used in many experiments in this institution. One gram finely ground rock phosphate (100 mesh) was shaken for

TABLE 13

The dissolution rate of a rock phosphate in a $KHSO_4$ solution at a pH of 2.00

TIME OF SHAKING	pH OF EXTRACT	P DISSOLVED FROM 1 GM. ROCK PHOSPHATE	TOTAL CONTENT OF P IN 1 GM. ROCK PHOSPHATE	P DISSOLVED IN THE $KHSO_4$ SOLUTION
minutes		*mgm.*	*mgm.*	*per cent*
1	2.42	40.2	135 5	30
3	2.50	40.5	135.5	30
5	2.57	44 4	135.5	33
15	2.71	50 3	135 5	39
30	2 71	53 6	135.5	39

varying periods of time with 400 cc. $KHSO_4$ at a pH of 2.00. P was determined after the Fiske-Subbarrow method (10).

DISCUSSION

It seems logical to assume that any acid solvent to be used in extraction of readily soluble phosphate from soils must possess an acidity of such a strength that the bulk of readily soluble phosphate in the soil sample is extracted; that the acid does not break up difficultly soluble soil phosphates appreciably; that the final acidity of the solvent is at a pH value such that re-precipitation of dissolved phosphate is prevented.

Readily soluble soil phosphate is defined as the amount of phosphate extracted, expressed as milligram P per 1,000 gm. of soil, by shaking 2 gm. of soil (20 mesh) for 5 minutes with a $KHSO_4$ solution of such a strength that the acidity of the filtered extracts is approximately 2.00. A solvent of this acidity has, for Ontario soils thus far tested, proved to fulfil the requirements mentioned.

Acid extraction of soil phosphate may be considered to be of the same value for estimating the solubility of phosphate as acid extractions of phosphatic fertilizer materials. A preliminary study on the solubility of synthetic phosphates and phosphatic fertilizer materials in a dilute acid solvent (pH 1.48) supports this assumption (20).

The data presented in table 10 and figure 1 show that the content of readily soluble soil phosphate is most clearly exposed in the series of extractions where the strongest solvent is used.

Plots 2 and 5, where the largest contents of readily soluble phosphate are found, have been treated with a ground rock phosphate, which, fixation experiments with phosphates have shown, is too difficultly soluble to be appreciably converted into the even more difficultly soluble soil phosphates (20). In the samples from plots 1, 3, and 4, in which the soil phosphate appears to be of a very difficultly soluble nature, the increased acidity of the solvents has not caused nearly so large an increase of dissolved phosphate as in the samples from plots 2 and 5.

Extractions of the samples from the Guelph profile (table 8) and from the fixation experiment (table 6) also reveal that the more difficultly soluble soil phosphates do not dissolve appreciably when the solvent is used at a pH of 2. It is interesting to note in this connection that Schloesing and de'Sigmond (34) have also found that difficultly soluble soil phosphates do not readily dissolve in this range of acidity. Studies on leached podzol profiles and very infertile soils from Ontario, have also proved this (21). The results from the Guelph profile (table 8) show that the solvent used in the Truog method is too weak to take care of the readily soluble bases in the samples from the B_2 and C horizons.

From table 2 it is seen that it is very important to extract readily soluble phosphate from soils at a pH value of the solvent, such that re-precipitation of the dissolved phosphate is prevented. This experiment also reveals that the amounts of phosphate extracted are proportional to the amounts of soil used when the pH value of the extracts is below approximately 3. In those cases where the pH values of the extracts are appreciably above 3, the amounts of phosphate extracted decrease considerably. The most plausible explanation is that some of the phosphate at first dissolved has been re-precipitated, as an excess of bases—and especially those of iron and aluminum—may cause almost complete precipitation of the phosphate ion above a pH of 3 (11). It must here be borne in mind that iron and aluminum do not need to enter into solution[9] in order to precipitate phosphate from a solution, as colloidal iron and aluminum hydroxides readily combine with phosphate in solution (11, 14). It is noteworthy that by acid extraction of the Welland soil (No. 2) the final pH of the extract was 3.28, only 3 mgm. of P per 1,000 gm. of soil was extracted, whereas by 1:500 water extraction, 9 mgm. P per 1,000 gm. of soil was extracted. The leaching experiments (table 11) in which a very thin layer of soil has been leached show that the acidity of the solvent greatly influences the amounts of phosphate leached from the soil.

The experiment with continuous leaching with $KHSO_4$ at a pH of 2 (table 12 and fig. 3) shows that 10 leachings remove practically all of the readily

[9] Practically no iron could be detected in the filtrates.

soluble phosphate, as determined at this acidity. Five minutes of shaking with the same solvent removes practically the same amount (table 9). All the experiments with solvents of different degrees of acidity reveal that the acidity of the solvent largely determines the amounts of phosphate extracted from soils containing much readily soluble phosphate, whereas from soils containing difficultly soluble phosphate the amounts of extracted phosphate do not increase to nearly the same extent. The $KHSO_4$ solvent, at a pH of 2.00, has, therefore, been chosen for the extraction of readily soluble soil phosphate from Ontario soils, because it is strong enough to expose the content of readily soluble phosphate in a soil sample, and to maintain a pH of approximately 2.00 in extraction of soil samples free from an appreciable amount of free carbonates. Besides this, difficultly soluble soil phosphates do not dissolve appreciably in this solvent. [See also Lohse and Ruhnke (21).]

Samples from surface and subsurface horizons in cultivated soils and A and B horizons in virgin soils very rarely cause an appreciable rise of the pH of the $KHSO_4$ solvent (pH 2.00).[10] But in extracting phosphate from samples of calcareous C horizons in virgin profiles and the lower horizons of agricultural soils, which often contain a considerable amount of free carbonates, the acidity of the $KHSO_4$ solvent often decreased considerably. When acid is being added to such a soil sample the primary reaction mainly taking place is

$$CaCO_3 + 2H^+ = Ca^{++} + \overbrace{H_2CO_3}^{H_2O + CO_2}$$

If the acid has only sufficient acidity to react with the carbonates present, practically no action of the acid on the phosphates can be expected to take place, and in some cases no phosphate has been extracted from such samples if the acidity of the solvent has not been corrected so as to yield an extract of pH approximately 2 (21).

Table 14 reveals the results of extraction of the C horizons of a Brown Forest soil—Kent profile (20, 21). The content of readily soluble phosphate in soil samples from C horizons containing free carbonates is usually relatively high (21).

Studies on calcareous soils in Arizona have shown that calcareous soils may contain a relatively large amount of phosphate extracted by dilute acid (Dyer and Truog methods), although they are phosphate deficient for crop growth, as a result of poor aeration (22). On soils with a high content of free carbonates in the upper layers, acid extraction of soil phosphate seems to be of limited value in determining phosphate deficiency for the crops grown there. Under humid conditions, however, where the carbonates are leached out of the upper soil horizons, extraction with dilute $KHSO_4$, as outlined in this paper, seems to give an *explicit index to the stock of readily soluble phos-*

[10] In the extraction of samples from soil types where no former experience has been obtained, acidity control of the extracts is advisable.

phate in the soil. As shown by Lohse and Ruhnke (21), such investigations should never be confined to the surface layer alone, because the subsequent layers often contain considerable amounts of readily soluble phosphate, which, no doubt, in many cases—especially in the case of deep-rooted crops—is utilized by the plants. It has been the experience in this institution that soil samples containing 30 mgm. or less of P per 1,000 gm. of soil, appear to indicate very marked phosphate deficiency. If two or three subsequent horizons are as low in extracted phosphate, the phosphate deficiency has been extremely marked. It should be mentioned that fertile soils for truck crops often contain as much as 500 to 600 mgm. of P per 1,000 gm. of soil and extremely fertile greenhouse soils run as high as about 1,000 mgm. P per 1,000 gm. of soil.

TABLE 14

Extraction of readily soluble phosphate from calcareous C-horizons from a Kent profile

HORIZON	pH*	CaO* (TOTAL)	CO_2* (TOTAL)	EXTRACTION WITH $KHSO_4$					EXTRACTION WITH HCl†	
				pH of extracts	P per 1,000 gm. soil ($KHSO_4$ solvent, pH 2 00)	Added gm. $KHSO_4$ to 400 cc. solvent, pH 2.00	pH of extract	P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil
		per cent	*per cent*		*mgm.*			*mgm.*		*mgm.*
C_1	7.77	14.32	12 16	7.11	44	2.000	2.00	270	2.04	300
C_2	7.87	10.10	8 72	6 63	11	1.200	2 03	282	2 03	295

* Analysis after Ruhnke (31).

† Buffered with KCl

No attempt has as yet been made to settle definite limits for an adequate phosphate supply for the different crops on different soil types. Such limits can only be determined by experience obtained by future collaborative laboratory and field experiments, and generalization will always be difficult because factors such as climate, crop species, and soil management have their influence upon crop production (3). It must be said, however, that control with field results (from Ontario) has shown that phosphate extractions of soils by the $KHSO_4$ method always have given a very useful index to the fertility of the soil with respect to phosphate.

In the light of the foregoing experimental data, there seems to be little that would warrant the use of the term *available* as applied to the amounts of soil phosphate extracted by acid extraction methods. By varying the experimental conditions, the amounts of phosphate extracted will also vary; and it is quite possible so to arrange the conditions that the extracted amounts of phosphate will be of approximately the same level as the amounts found in crops. However, in order to achieve this result, it will be necessary, if acid

extraction methods are used, to use either a *narrow* soil to solvent ratio[11] or a *very weak* acid solvent. Tables 2 and 8 indicate, however, that neither of these conditions is desirable and that both may lead to fallacious results. If solvents weaker than pH 2 were used, leachings through a *very thin layer of soil* would, no doubt, be the best, as it would, in this way, be possible to control re-precipitation of dissolved phosphate. Experimentation on this point, however, has not yielded very satisfactory results.

CONCLUSIONS

Since extensive studies of typical soils and also comparison with several other methods have shown that a satisfactory index to the amount of readily soluble phosphate in soil samples is obtained by extraction with dilute $KHSO_4$ at a pH of 2.00, the following extraction method has been adopted:

Procedure for extraction of readily soluble phosphate from soils by means of dilute $KHSO_4$ at a pH of 2.00 ($KHSO_4$-method). Place 2 gm. of air-dried and sieved (20-mesh) soil in a 750-cc. Erlenmeyer flask, add 400 cc. of a $KHSO_4$ solution at a pH of 2.00 and shake 5 minutes. (Hand shaking is sufficient.) Filter at once, by using suction, through a high quality filter paper on a Buchner funnel with an inside diameter of 9½ cm. Rapid filtering—only a few minutes—is necessary. The supernatant liquid only should be poured on the filter. Discard the first 50 cc. of the filtrate, and if the filtrate is still not clear, discard the filtrate until a clear filtrate is obtained. In rare cases, a double layer of filter paper may be necessary. It is advisable to wash the filter paper by sucking about 50 cc. of water through it in two or three portions before filtering the extract. For the colorimetric determination of the phosphate, the Deniges method, as worked out by Truog and Meyer (37) is suitable, even though other colorimetric methods can also be employed (12, 13). In the most ordinary cases, 50 cc. of the filtrate can be used directly for the colorimetric determination without dilution or concentration. As the $KHSO_4$ solvent possesses a relatively high acidity, adjustment for this is most easily done in preparing the ammonium-molybdate-sulfuric acid solution if the Truog and Meyer method is used. It can also be done by means of dilute NH_4OH. According to Chapman (4), it is desirable to add the crude extract *to* the acid molybdate mixture so as to avoid formation of persistent silico-molybdate if dissolved silica should be in the solution. It is most convenient to mix the molybdate solution and the extract in 100-cc. Erlenmeyer flasks. If As^V or Fe^{III} are present, they must be reduced by means of a reductor or H_2S (4).

Only high-grade, previously tested filter papers (4, 36, 37) should be used. Suitable filter papers for this purpose are *Whatman No. 42, C. Schleicher and Schull, No. 589, White Kibbon, and Berzelius No. O. B.* It is also important to run a blank determination every time new reagents are put into use and the extraction should be run in *duplicate.*

When dealing with calcareous soil samples, the pH of the extracts should always be controlled. If the pH of the extract has changed materially from 2.00, adjustment of the $KHSO_4$ solvent with solid $KHSO_4$ should be made. How this adjustment is made is shown by the following typical example:

---

[11] When dissolved phosphate is to be determined directly in the crude extracts, a wide soil to solvent ratio is also preferable, because dissolved silica, organic matter, iron, etc., which, in appreciable concentrations interfere with the colorimetric determinations, thereby are diluted.

By extracting a calcareous soil sample from the C_3 horizon of the Kent profile (see table 14), the pH of the extract was 6.63 and only 11 mgm. P per 1,000 gm. of the soil was extracted. The concentration of the $KHSO_4$ in 400 cc. of solvent necessary to combine with the bases in the sample in order to obtain a pH of approximately 2.00 in the extract, was determined by graphical interpolation as follows: 2 gm. of soil were weighed out in each of two Erlenmeyer flasks. One and two grams, respectively, of solid $KHSO_4$ were dissolved in two 400-cc. portions of $KHSO_4$ at a pH of 2.00 and the solutions were added to the two Erlenmeyer flasks.[12] These were shaken 5 minutes and filtered as described in the foregoing. The pH values in the extracts were determined on the quinhydrone electrode, and the $KHSO_4$ concentrations and the pH values were plotted in a graph (fig. 4). By interpolation of the concentration of $KHSO_4$ in the extract necessary in order to obtain a pH of approximately 2.00, this amount was found to be 3.292 gm. $KHSO_4$. By repeating the extraction with this concentration of

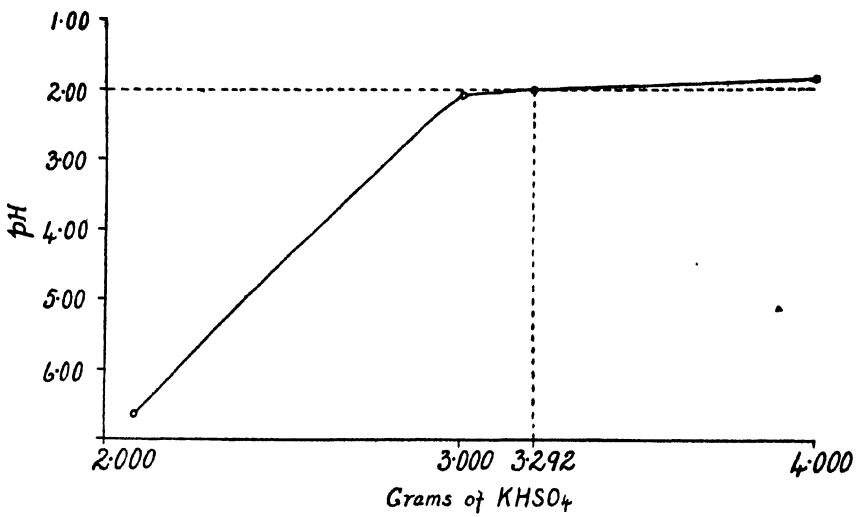

FIG. 4. INTERPOLATION OF THE AMOUNT OF $KHSO_4$ TO BE ADDED TO THE $KHSO_4$ SOLVENT IN ORDER TO FINISH AT A PH OF 2.00 IN EXTRACTING A CALCAREOUS SOIL SAMPLE

$KHSO_4$ in the solvent, a pH of 2.03 was obtained in the extract, and 282 mgm. P per 1,000 gm. of soil was found. The following table and figure 4 show how this operation is done.

$KHSO_4$ IN 400 CC. SOLVENT	pH OF EXTRACTS	P PER 1,000 GM. SOIL
gm.		*mgm.*
2.092	6.63	11
3.092	2.09	..
4.092	1.85	..
3.292	2.03	282

With a little experience, this operation takes very little time.

When working with the extraction of readily soluble phosphate from soils, acidity control is absolutely necessary for the interpretation of the results. From the determination of the

[12] If extracting a large number of calcareous soil samples, the use of two stock solutions of these concentrations of $KHSO_4$ could be employed.

pH values in the *extracts*, the quinhydrone electrode is rapid and, from the experience obtained so far in this laboratory, is also reliable.

The whole operation in determining readily soluble phosphate in a soil sample, extraction, filtering, and colorimetric determination can be done within half an hour, and a routine analyst can determine readily soluble phosphate in about 20 soil samples a day.

SUMMARY

Readily soluble soil phosphate is defined as the amount of phosphate extracted—expressed as milligrams P per 1,000 gm. of soil—by shaking 2 gm. soil (20 mesh) for 5 minutes, with a $KHSO_4$ solution of such a strength that the acidity in the filtered extracts is approximately 2.00.

An acid solvent of this strength has proved to be strong enough to extract the readily soluble phosphate in a soil sample; it does not break up the difficulty soluble soil phosphate appreciably; and the acidity of the extracts is high enough to prevent re-precipitation of dissolved phosphate.

The readily soluble phosphate in a soil sample is adequately removed by 5 minutes of shaking.

A method for extraction of readily soluble phosphate from soils by means of dilute $KHSO_4$ at a pH of 2.00 is described.

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STUDIES ON READILY SOLUBLE PHOSPHATE IN SOILS: II. THE VERTICAL DISTRIBUTION OF READILY SOLUBLE PHOSPHATE IN SOME REPRESENTATIVE ONTARIO SOILS[1]

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It has been well established, that the roots of most cultivated plants, under natural conditions, penetrate into the lower horizons of the soil (20, 21). Undoubtedly, the plants do, in many cases, absorb nutrients from the lower soil horizons. Because of this, investigations on readily soluble nutrients from soils should not be restricted to the surface or cultivated layer alone, but should include all the subsequent horizons.

The purpose of this paper is to present some typical data on the vertical distribution of readily soluble phosphate in some typical soil horizons of both virgin and cultivated soils from Ontario, in the brown forest and podzol groups. The reason for publishing these preliminary results is partly for the purpose of giving some further data supporting the extraction method described elsewhere (9), and partly to call attention to the striking results obtained by extraction of readily soluble soil phosphates at a controlled pH value.

REVIEW OF LITERATURE

Only very few investigations reported in the literature are of the same nature and scope as the investigations presented here.

A large number of references deal with the distribution of the total amount of phosphorus in the different horizons of soils (1, 2, 6, 7, 10, 13, 14, 23, 24). Jenny (7) has given an excellent description of the distribution of the different elements (total amounts) in typical regional profiles in the United States. With respect to the total phosphorus content and its distribution in the profile, the analysis given for the podzol profile (7, p. 175) is most striking. The A_2 horizon of maximum leaching is almost devoid of this element. It must be admitted, however, that the total analysis gives very little information in regard to the nature and the movement of the soil phosphates in the horizons.

In studies of the water-soluble constituents in loess soils in Nebraska by Upson and Calvin (19), no regularity in the distribution of water-soluble phosphates was found. In studies of soils from the same region by Alway and Isham (1), it was shown that the amount of soil phosphates dissolved in 1 per cent citric acid extracts in the humid eastern area increased markedly from the first to the sixth foot, whereas in the most westerly arid portion a marked

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[3] Published with the permission of the president.

decrease from the surface downward was found. It is important to note that the presence of carbonates in the subsoils from the western arid area interfered with the citric acid extraction. When carbonates were destroyed before extraction, the sixth foot horizon contained as much phosphate soluble in 1 per cent citric acid as did the first foot horizon.

Dunnewald (4) using 0.001 *N* H_2SO_4 has studied the distribution of "available" phosphorus in some Wyoming soil profiles. He concluded that "available" phosphorus decreases in the lower soil zones of all the profiles studied, as compared with the surface zones, where most of the organic matter has accumulated. This was true in basic soils as in those with acid reaction. He observed that in the basic soils the amount of lime increases in the lower zone to such an extent that the action of the 0.001 *N* acid is entirely neutralized, resulting in lowered values for "available" phosphorus. In a more recent paper (5) Dunnewald concludes that "available" phosphorus is more rapidly lost from the surface from grass soils than from podzolized timber soils.

Nemec (12) found that the humus layer in forest soils is the most abundant source of easily soluble phosphate, and in the lower layers there is a general parallelism between the citric acid solubility of soil phosphate and the humus content. The humus layer under pines is relatively low in citric acid-soluble phosphate. Much of the damage caused by removal of the débris of leaves for litter may be ascribed to the resulting decrease in the content of "available" phosphate in the soil.

Spurway (15) found, in Michigan soils, that fertilized muck soils retained nearly all of their water-soluble phosphate in the plowed portion, but in fertilized mineral soils, a downward penetration of water-soluble phosphate took place.

Stephenson and Chapman (16) working with California soils, have shown that readily soluble phosphate in the soil can penetrate appreciably below the surface foot in light to medium textured soils. Little or no penetration seems to take place in very heavy sbils. The experiments indicated also that readily soluble phosphate from manure moves readily through the soil. Midgley (11) found that superphosphate, where applied as a surface dressing, moves downward very slowly. This downward movement is greatly influenced by different fertilizer salts.

In studies on Danish heath soils (podzols) Weis (22) found that they were very poor in phosphate extracted with nitric acid. In heavily podzolized sandy profiles, practically no phosphate could be extracted. An estimation of the distribution of the inorganic colloids showed that the largest amounts of inorganic colloids were accumulated in the B horizon.

In studies on southern Ontario soil profiles, Ruhnke (14) also has found that an accumulation of colloidal matter takes place in the B horizons.

The distribution of the fine material in soils might have great bearing upon the distribution and the nature of phosphates, as the phosphate content in the soil, after Lauterberg (8), is largely concentrated in the finer particles (less than 0.05 mm.).

EXPERIMENTAL METHODS

Sampling was done on the basis of natural horizons wherever possible. In the cultivated clayey soils, however, where it was difficult to distinguish the horizons, samples were taken at arbitrary depths corresponding to the changes in color and texture. The samples were air-dried on arrival at the laboratory, and when air-dried, passed through a 20-mesh sieve.

Reaction. The pH values of the soil samples were determined in 1:1 suspensions (CO_2-free distilled water used). In most cases the quinhydrone electrode was used, but in samples where the alkalinity was too high, colorimetric determinations were made.[4]

[4] All the samples have also been tested for their approximate pH value by a simple colorimetric test ranging from a pH of 4.4 to a pH of 7.6.

Readily soluble phosphate was extracted by means of dilute $KHSO_4$ at a pH of 2.0 (9); 2 gm. of air-dried soil sieved through a 20-mesh sieve was shaken 5 minutes with 400 cc. of a $KHSO_4$ solution at a pH of 2.0. Immediately after the elapse of 5 minutes the supernatant suspension was filtered rapidly through a 9½-cm. Buchner funnel. The phosphate content in the extracts was determined by the Deniges method, as worked out by Truog and Truog and Meyer (18), and somewhat modified by Chapman (3).

The term *readily soluble soil phosphate* refers to the amount of phosphate extracted from a soil sample by this procedure.

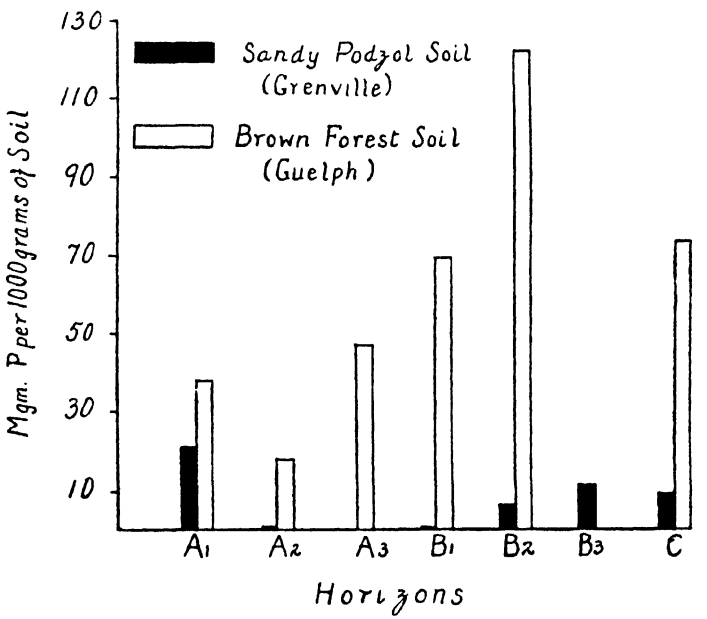

FIG. 1. THE DISTRIBUTION OF READILY SOLUBLE SOIL PHOSPHATE IN A TYPICAL SANDY PODZOL SOIL AND IN A TYPICAL BROWN FOREST SOIL

EXPERIMENTAL DATA

The experimental data obtained in the investigations dealt with in this paper are presented in tables 1, 2, 3, and 4. Figure 1 shows the distribution of readily soluble phosphate in a typical acid sandy podzol soil and in a typical alkaline brown forest soil profile.

DISCUSSION

Virgin podzol soils (table 1). The profiles are acid in all horizons. The relatively high pH value, 6.91, in the A_1 horizon from the Caledon profile is most likely due to material transferred up to the surface from a deeper layer, as this profile was sampled from a road cut.

The *sandy* podzols are distinguished by their very low content of readily soluble phosphate. It is interesting to note in this connection that Weis (22) found almost no phosphate by acid extraction of podzolized sandy heath profiles in Denmark. It will be seen that the surface layer contains the highest amount of phosphate. Dunnewald (4) found the same in acid profiles.

The *clayey* podzols are much richer in readily soluble phosphate in all horizons, although they are acid in reaction, and they have been vigorously leached.

TABLE 1

Reaction and readily soluble phosphate in virgin podzol soils

LOCATION	HORIZON	DEPTH	pH	P PER 1,000 GM. SOIL	REMARKS
		inches		*mgm.*	
1. South Gower Grenville	A_1	0–4	5.35	21	Sandy podzol developed on outwash plain material under predominantly coniferous forest with some hardwoods
	A_2	4–7	5.02	None	
	B_1	7–16	4.77	None	
	B_2	16–26	5.47	6	
	B_3	26–36	5 67	11	
	C	36–44	5 77	9	
2. Peel County Caledon Highland	A_1	0–3	6 91	17	Sandy loam podzol developed on moraïnic material covered with mixed forest
	A_2	3–4	4.83	7	
	A_3	4–7	5.91	None	
	B_1	7–13	6.10	6	
	B_2	13–16	6.10	11	
	C	16–30	5.97	14	
3. Warren Sudbury	A_1	.....	5.63	53	Clayey podzol on smooth till plain area. Sudbury district, northern Ontario
	A_2	.....	5 49	36	
	B_1	.....	5.55	44	
	B_2	.....	5 63	168	
	B_3	.....	6.12	225	
	C	.....	6.53	540	
4. Cochrane district Monteith	A_1	.....	5.81	125	Clayey podzol on gently rolling till plain area
	A_2	.....	4.70	19	
	B_1	.....	5.18	72	
	B_2	.....	5.50	204	
	C	.....	6.64	285	

The high content of readily soluble phosphate in the parent material in the clayey podzols is remarkable. Undoubtedly, the contrast between clayey and sandy podzols is partly explained by the great difference in the character of the parent materials from which these soils have developed.

Virgin brown forest soils (table 2). The content of readily soluble phosphate throughout the profiles is of the same magnitude level as found in the clayey podzols. All the soils presented are typical brown forest soils.

In the pronounced acid A horizons of the Kent profile (no. 3) the content of

readily soluble phosphate is very low in spite of the fact that the content in the parent material is high. Numbers 1 and 3 have the highest content of readily soluble phosphate in the parent material, numbers 2, 4, 5 in the B horizons. An accumulation of readily soluble phosphate in the B horizons may be due to the relatively high content of colloidal material found in this horizon (8, 14, 22). A high content of readily soluble phosphate in the parent material is explainable

TABLE 2

Reaction and readily soluble phosphate in virgin brown forest soils

LOCATION	HORIZON	DEPTH	pH	P PER 1,000 GM. SOIL	REMARKS
		inches		*mgm.*	
1. Beverly Wentworth Co.	A_1	0–5	6 98	27	Well-drained silt loam, developed on lacustrine deposit in glacial lake plain. Cover grass
	A_2	5–9	7 03	16	
	B	9–20	7.26	342	
	C	20–30	8.02	516	
2. Caledon East Peel Co.	A_1	0–3	7.69	27	Well-drained loam, developed in till plain area. Cover grass
	A_2	3–11	7 14	120	
	B	11–22	7 27	253	
	C	22–30	8.13	140	
3. MacGregor Creek Kent Co.	A_1	0–4	5 90	9	Moderately well-drained silt loam, developed on lacustrine deposit in glacial lake plain. Cover grass
	A_2	4–8	5.77	10	
	A_3	8–13	5 51	8	
	B_1	13–22	6 28	63	
	B_2	22–28	7 75	218	
	C_1	28–33	7.77	270	
	C_2	33–40	7 87	282	
4. Guelph Wellington Co.	A_1	0–5	7 94	38	Well-drained upland silt loam, on limestone drift. Cover grass
	A_2	5–11	7 68	18	
	A_3	11–19	7.68	47	
	B_1	19–28	7 66	69	
	B_2	28–36	8 27	122	
	C	36–42	8 01	73	
5. Beverly Wentworth Co.	A_1	0–5	7 33	20	Well-drained upland loam on limestone drift. Cover grass
	A_2	5–12	7 23	51	
	B	12–23	7.81	160	
	C	23–34	8.16	80	

from the fact that this horizon has a very high content of Ca. When such C horizons are being extracted with dilute acids, large amounts of dissolved Ca can be detected, whereas the extracts from A and B horizons contain only small amounts of dissolved Ca.

Cultivated brown forest soils (table 3). The striking feature in this group is the high content of readily soluble phosphate in the lower horizons. Numbers

TABLE 3

Reaction and readily soluble phosphate in cultivated brown forest soils

LOCATION	DEPTH	pH	P PER 1,000 GM. OF SOIL	REMARKS
	inches		*mgm.*	
1. Pain Court Kent Co.	0–10	7.38	183	Very productive dark brown soil. Corn, sugar beets, alfalfa, beans, wheat. Never fertilized
	10–18	8.00	204	
	18–28	8.12	213	
2. Ringold Kent Co.	0–6	7.28	142	Very productive black soil. Corn, sugar beets, alfalfa, grain, grown on drained areas. Never fertilized
	6–12	7.16	132	
	12–20	7.17	186	
	20–30	7.41	255	
3. Caledon E. Peel Co.	0–8	7.91	70	Gray-brown loam. Alfalfa area. Stand of alfalfa excellent—drainage good
	8–16	8 16	450	
	16–24	8.15	378	
4. Bolton Peel Co.	0–8	5.70	29	Gray-brown clay loam—typical alfalfa soil. Peel Co. Received 175 pounds 2-8-4 fertilizer, 1931. No previous fertilizer treatment
	8–16	7.35	277	
	16–25	8 13	550	
5. Brampton Peel Co.	0–8	6.08	14	Gray-brown clay loam-14-year-old alfalfa sod—plants vigorous but stand thinning out. Farmyard manure used 10 years ago, but no commercial fertilizer
	8–18	5.82	114	
	18–27	6.39	325	
6. Rainham Haldimand	0–6	5.18	13	Light gray-brown clay-rotation pasture. Canadian bluegrass. Farmyard manure in rotation but no commercial fertilizer ever used. Cultivated about 100 years
	6–14	6.46	30	
	14–22	8.07	475	
	22–30	7.97	420	
7. Charing Cross Kent Co.	0–8	5.89	17	Light gray-brown clay loam. Reverted to permanent pasture. Canada bluegrass. Little white clover. Never fertilized
	8–16	5.80	10	
	16–24	6.08	9	
	24–32	7.40	123	
	32–40	7.63	280	
8. Rainham Haldimand	0–8	4.85	None	Similar to Rainham soil above. Superphosphate gives very marked results where used on wheat on this farm
	8–16	4.89	10	
	16–24	6.87	200	
	24–32	7.78	410	

1, 2, and 3 are alkaline in all horizons and they have also a very high content of readily soluble phosphate. They are all very fertile soils. Numbers 1 and 2 are typical sugar beet soils from Kent County. Numbers 4 and 5 are typical alfalfa soils from Peel County. It will be seen that they are very much ex-

hausted of readily soluble phosphate in the surface. Since alfalfa can grow well in spite of that, it is apparently due to the fact that the plants can get their supply of phosphate from the lower horizons. From experience in this laboratory, a content of 14 mgm. and 29 mgm. P per 1,000 gm. of soil, respectively, as extracted by the $KHSO_4$-method, is so low that it would mean failure of the alfalfa crop if no other sources of phosphate were available.

For the start of a new sown crop on soils, as nos. 4 and 5 (especially one of short duration and quick growth), phosphate fertilization seems to be advisable.

Numbers 6, 7, and 8 represent cultivated soils, with an extreme deficiency of readily soluble phosphate. The cover on the pasture where sample 7 was taken consisted of a very poor stand of Canada bluegrass. The livestock on the

TABLE 4

Extraction of readily soluble soil phosphate in calcareous C horizons ($KHSO_4$ method)

PROFILE	FIRST EXTRACTION		FINAL EXTRACTION		
	pH in extract	P per 1,000 gm. soil	$KHSO_4$ added*	pH in extract	P per 1,000 gm. soil
		mgm.	*gm.*		*mgm.*
No. 4 Table 1	2.20	180	0.850	2 03	285
No. 2 Table 2	5 50	9	1.400	2 05	140
No. 3 Table 2 C_1	7 11	44	2 000	2 00	270
No. 3 Table 2 C_2	6 63	11	1 200	2 03	282
No. 4 Table 2	2 33	56	0 700	2 02	73
No. 1 Table 3	2 21	136	0.900	2 03	213
No. 3 Table 3	3 01	118	1 000	2 02	378
No. 4 Table 3	3 40	46	1 100	2 04	550
No. 6 Table 3	5 58	None	1 500	2 01	420
No. 8 Table 3	5 73	8	1 500	2 04	410

* The amount of solid $KHSO_4$ added in order to finish the extraction at a pH of approximately 2.0 is determined by interpolation on the basis of two extra extractions with higher acidity of the solvent.

farms where samples 6 and 8 were taken suffered severely from mineral deficiency. It is worthy of note that in these soils, where phosphate deficiency is so marked, a low level of readily soluble phosphate is found in both surface and subsurface horizons.

Summarized, it can be stated for these representative cultivated soils, that nos. 1, 2, and 3, represent soils not phosphate deficient; nos. 4 and 5 represent soils where deep rooted crops, once established, will have an adequate phosphate supply; and nos. 6, 7, and 8 represent soils of extreme phosphate deficiency.

As far as samples from cultivated soils are concerned, the few examples presented here are taken as typical. Numerous samples from cultivated soils have been extracted for readily soluble phosphate by the $KHSO_4$-method up to the present date. Experience thus obtained, in comparison with other methods and field experience, have shown that a marked phosphate deficiency

in Ontario soils is found where subsequent layers have contained below 30 mgm. P per 1,000 gm. of air-dried soil. What limits one should propose as optimal, or rather as rational, for the different crops, on different soil types, under different climatic conditions, and by different systems of field management, can only be decided by future collaborative experimental work in the laboratory and in the field.

Table 4 gives the results of extractions of calcareous C horizons.

CONCLUSIONS

These investigations show conclusively that surface sampling alone, for the purpose of determining soil fertility, is inadequate. Soils 4 and 5 from the alfalfa section in Peel County are striking examples in this respect.

The importance of extracting all the horizons at the same pH value in the extracts is apparent. For the case of calcareous C horizons, no idea as to the content of readily soluble phosphate would have been obtained if they had not been extracted at the same pH value as the A and B horizons.

Weaver (20) and Weaver and Bruner (21) have conclusively shown, in their extensive studies on the root development of cultivated plants, that deeply rooted plants can benefit by the plant food contained in the lower soil horizons. It thus becomes apparent that the high amounts of readily soluble phosphate, as found in the lower soil horizons in a number of soils described in this paper, are of great importance for the supply of phosphate to deeply rooted plants, such as alfalfa, sweet clover, and sugar beets. The results from the profiles from the alfalfa section in Peel County (nos. 4 and 5, table 3), represent striking examples in this respect.

There might be some doubt as to the ability of the plants to utilize the phosphate sources in the alkaline calcareous parent material,[5] and when phosphate from the lower horizons can be utilized by deeply rooted plants, it is most likely that this absorption of phosphates takes place from the B horizon.

It is a question of great importance in soil fertility studies, and it deserves further study.

SUMMARY

Readily soluble soil phosphate, extracted by 5 minutes of shaking with a $KHSO_4$ solution at a pH of 2.0, varies much in vertical distribution. Therefore, surface sampling alone is inadequate.

[5] Recent investigations of McGeorge and Breazeale (Tech. Bul. 35 and 36, Univ. of Ariz., Agr. Exp. Sta., 1931) have shown, that highly calcareous soils may have a high content of readily soluble phosphate, as determined by the methods of Dyer and Truog, in spite of the fact that these soils were phosphate deficient from an agricultural standpoint. The same authors have also shown that a high content of bicarbonate checks the phosphate absorption of plants. It thus becomes apparent that acid extractions of phosphate from soils high in carbonates (in arid regions) are of a very limited value. Soils from the humid regions, however, where the carbonates are leached out of the layers where the plants most likely absorb the phosphate acid extractions, as described in this paper, give a very useful index to the stock of readily soluble phosphate in a given soil sample.

In determining the vertical distribution of readily soluble soil phosphate, extraction at the same pH value in the extracts from all the horizons, is desirable. The extracts must also be at a pH value where re-precipitation of dissolved phosphate is prevented.

Sandy podzol soils seem to be very low in readily soluble phosphate in all horizons, the parent material included.

Clayey podzols and brown forest soils contained large amounts of readily soluble phosphate in the B horizon and in the parent material.

Cultivated soils of the brown forest group contained, in all cases, considerable amounts of readily soluble phosphate in the lower horizons.

Extractions of profile samples from typical cultivated brown forest soils, well known as to their fertility, indicate that the extraction method ($KHSO_4$ method) used gives a good index to the fertility of these soils, as far as their phosphate supply is concerned.

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FURTHER STUDIES ON NITROGEN ABSORPTION FROM CULTURE SOLUTIONS: II. BUCKWHEAT[1]

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The investigations described in the following pages deal with a quantitative study of the absorption of nitrogen by the roots of buckwheat plants (*Fagopyrum esculentum* Moench.) from culture solutions in which they were grown. This paper is the second of two publications dealing with this problem. The experimental work of the present study with buckwheat was carried out in connection with the earlier work with oats (5), and in precisely the same manner and by the same methods, with only such modifications in treatment and handling as are necessitated by the differences in the forms and the relative duration of the life cycles of the two species.

The experimental procedure, including methods of growing the plants, the transfer of cultures for the absorption tests, and the methods of chemical analyses were described in detail in the earlier paper (5), therefore need not be repeated here. Any deviation from the experimental procedure dealt with in the paper just cited will be described in connection with the presentation of experimental results.

As has been stated in the earlier publication, the purpose of these investigations was primarily to determine the relative activity of the plants with respect to the absorption of nitrogen in the two forms, NH_4 and NO_3, by the roots of plants at various stages throughout the active life cycle, when both these forms of nitrogen are simultaneously present in the culture solution and available to the plants.

EXPERIMENTAL RESULTS

Preliminary experiments

In connection with some preliminary experiments with oats (5), similar experiments were carried out with buckwheat. As before, some of the data obtained from these preliminary experiments, which were carried out without continuous renewal of the culture solution during the test intervals, will be presented for the purpose of comparing the results with those obtained from the later experiments in which use was made of the method of continuous solution renewal at constant rates (4) throughout all the absorption intervals.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station department of plant physiology.

The plants were grown in the modified Tottingham solution $T_1R_3C_5$ (1) to the age of 18 days in 2-quart fruit jars of colorless glass, with continuous solution renewal. The plants were then transferred, as previously described (5) from the 2-quart jars to pint jars containing 500 cc. of the test solution, where they remained for the time of the test interval without continuous flow of the solution through the culture jar. At the end of the test interval the plants were returned to the original 2-quart culture jars, after the roots had been rinsed free from adhering solution with distilled water, the rinse water being retained with the solution for analysis to determine the quantities of nitrogen removed by the plants. Here the plants were grown as before until the next experimental period. This procedure was continued through the growth period of 58 days.

Since the plants were not harvested at the end of each test interval, dry weight yields were not obtained upon which to calculate rate absorption on the basis of nitrogen absorbed from solution per unit of dry plant material per unit of time. In these preliminary experiments, therefore, all results were calculated only on the quantity basis of nitrogen in the form in question absorbed from a fixed quantity of solution, in milligrams per culture per hour. These data are presented in table 1. Here again, as in the experiments with oats, 12 test intervals were employed during each experimental period. These ranged in time from 8 hours as the minimum to 96 hours as the maximum time during which the roots of the plants were exposed to the test solution. Each horizontal section of table 1 presents the data obtained during an experimental period of 96 hours. In making the absorption tests, duplicate cultures were employed and all analyses were made in duplicate; hence each value in the table except those in the last line of each horizontal section, represents the average of four determinations. Since duplicate cultures comprising three plants each were employed for each of the 12 test intervals, each of the values in the last line of the horizontal sections, marked *average*, represents the final average of 48 determinations involving 24 cultures and 72 plants.

The final average values for all the experimental periods as given in table 1 were plotted to form the graphs of figure 1. As indicated by the graph representing the course of quantity absorption of nitrogen as NH_4 per culture per hour during the growth cycle investigated, the maximum intake of nitrogen in this form occurred at the 18-day period, with rapid decline to a very low value at 30 days and thereafter to the stage of maturity and the formation of ripe seeds.

The graph representing the course of quantity absorption of nitrogen as NO_3 begins with relatively low values, rises to a maximum at 50 days, and then rapidly declines with approaching maturity. Thus, maximum quantity absorption of nitrogen as NO_3 occurs at a relatively late period in the growth cycle, whereas the maximum quantity absorption of nitrogen as NH_4 occurs early in the cycle, at the beginning of the blossoming period.

It will be observed that maximum quantity absorption of nitrogen as NO_3 is

TABLE 1

Quantity absorption of nitrogen as NH_4, NO_3, and total nitrogen per culture by buckwheat plants during absorption intervals ranging in time from 8 hours to 96 hours, at different stages of development in the life cycle

AGE OF PLANTS	ABSORPTION INTERVALS	QUANTITY OF NITROGEN ABSORBED PER CULTURE					
		As NH_4		As NO_3		Total nitrogen	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
18	8	3 764	0.471	−0 178	−0 022	3.586	0.449
	16	5 839	0.365	−0.799	−0 050	5.040	0.315
	24	5.426	0.226	−0.711	−0 030	4.715	0.196
	32	5.952	0.186	3 594	0 112	9 546	0 298
	40	7.439	0 186	3 594	0 090	11 033	0 276
	48	7 527	0 157	5.863	0 122	13 390	0.279
	56	7.089	0.127	6 573	0 117	13 662	0.244
	64	7.264	0.114	7.106	0 111	14.370	0.225
	72	5 962	0 083	7 595	0 105	13 557	0 188
	80	5.426	0 068	10.393	0 130	15 819	0.198
	88	6.127	0 070	10 393	0 118	16.520	0 188
	96	6 714	0 070	9 060	0 094	15 774	0 164
Average			0 177		0 075		0 252
30	8			1 534	0 192	1.534	0 192
	16	−0 120	−0 008	2 941	0.184	2 821	0.176
	24	−0 297	−0 012	4 459	0.186	4 162	0.174
	32	0 221	0 007	3 718	0 116	3 939	0.123
	40	0 332	0 008	5 903	0.148	6 235	0 156
	48	0 703	0 015	7.581	0 158	8 284	0.173
	56	1 295	0 023	7 834	0 140	9.129	0.163
	64	1 444	0 023	8 198	0 128	9 642	0 151
	72	2 853	0 040	8 272	0 115	11 125	0 155
	80	3 333	0 042	7 977	0 100	11 310	0.142
	88	4 296	0 049	8 162	0 093	12 458	0 142
	96	4 296	0 045	8 656	0 090	12 952	0 135
Average			0 019		0 138		0.157
42	8	−0 410	−0 051	4 525	0 566	4.115	0.515
	16	−0 602	−0 038	5.434	0 340	4 832	0.302
	24	−0 602	−0 025	5 842	0.243	5.240	0 218
	32	0 121	0 004	6 251	0.195	6.375	0.199
	40	0 396	0.010	6 673	0.167	7 069	0 177
	48	1.106	0 023	7 785	0.162	8 891	0.185
	56	1 699	0.030	8 360	0 149	10 059	0 179
	64	1 890	0 030	9.012	0.141	10.902	0 171
	72	2.274	0 032	9.702	0 135	11 976	0.167
	80	2.312	0.029	10 392	0.130	12 704	0 159
	88	2.235	0.025	10.734	0 122	12.969	0.147
	96	1.697	0.017	11.006	0.115	12 685	0 132
Average			0 027		0 205		0 213

TABLE 1—*Concluded*

AGE OF PLANTS	ABSORPTION INTERVALS	QUANTITY OF NITROGEN ABSORBED PER CULTURE					
		As NH_4		As NO_3		Total nitrogen	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
50	8	−0.395	−0.049	4 162	0.520	3.767	0.471
	16	−0.488	−0.031	5.778	0.361	5.290	0.330
	24	−0.244	−0.010	6.952	0.290	6.708	0.280
	32	0.000	0.000	6.757	0.211	6.757	0.211
	40	0.242	0.006	7.883	0.197	8.125	0 203
	48	1.076	0.022	8.519	0 177	8.595	0.199
	56	0.981	0.018	8.568	0.153	9.549	0.171
	64	1.322	0 021	9.106	0.142	10.428	0.163
	72	1.764	0.025	9.106	0.126	10.870	0.151
	80	1.373	0.017	9.694	0.121	11.067	0.138
	88	1.960	0.022	10.183	0.116	12 143	0.138
	96	2.058	0.021	10.183	0.106	12.241	0.127
Average			0.005		0 210		0.215
58	8	−0.243	−0.030	1 064	0.133	0.821	0.103
	16	−0.488	−0.031	1.162	0.073	0.674	0.042
	24	0.099	0.004	1.455	0 061	1.554	0.065
	32	0.639	0.020	2.139	0.067	2.778	0 087
	40	1.472	0.037	2.579	0.064	4.051	0.101
	48	1.374	0.029	3.068	0.064	4.442	0 093
	56	1.570	0.028	3 605	0 064	5.175	0.092
	64	1.565	0.024	3.556	0 056	5.121	0 080
	72	1.766	0.025	4 192	0.058	5.958	0.083
	80	1.472	0.018	5.249	0 066	6.721	0.084
	88	1.962	0.022	5.364	0.061	7.326	0.083
	96	1.962	0.020	6.049	0 063	8.011	0.083
Average			0.014		0.069		0.083

somewhat higher than maximum quantity absorption as NH_4. However, since the former occurs at a time when the plants are relatively large and the latter when the plants are small, the NO_3-rate of absorption at the point of maximum quantity absorption is very much lower than the NH_4-rate at the point of maximum quantity absorption. This will be clearly brought out in connection with the presentation of the data on rate absorption calculated on the basis of milligrams of nitrogen absorbed per gram of dry plant material per hour.

Quantity absorption of total nitrogen as indicated by the course of the upper graph of figure 1, is determined while the plants are young by quantity absorption of nitrogen as NH_4, and during the late growth phases by quantity absorption of nitrogen as NO_3. This is in direct agreement with the results obtained

with oats. It also harmonizes with the work of Naftel (2), who found that cotton seedlings before they were from 3 to 5 weeks old absorbed more NH_4 nitrogen than NO_3 nitrogen; but after this age more NO_3 nitrogen was absorbed in a given time than NH_4 nitrogen. This graph also indicates a secondary maximum point for quantity absorption of total nitrogen, which occurs late in the growth cycle (50 days) and is determined by quantity absorption of nitrogen as NO_3.

As in the studies with oats, so again in these preliminary experiments with buckwheat, it is important to point out the fact, as indicated by the data of

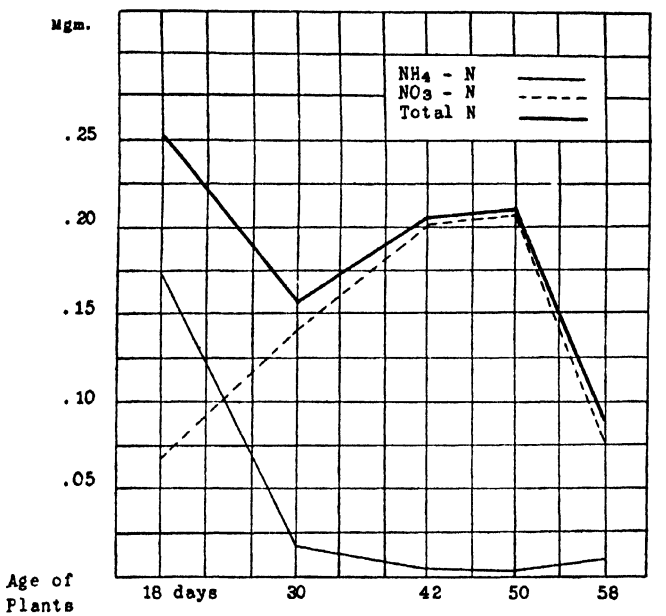

FIG. 1. GRAPHS REPRESENTING QUANTITY ABSORPTION OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN IN MILLIGRAMS PER CULTURE PER HOUR AT DIFFERENT PERIODS IN THE ACTIVE LIFE CYCLE OF THE BUCKWHEAT PLANT

table 1, that without continuous renewal of the culture solution during the test intervals, quantity absorption per culture per hour decreases very markedly with increase in the time duration of the absorption interval, when the roots are exposed to a fixed, relatively small quantity of solution (500 cc.). This influence of the duration of the test intervals upon absorption is negligible only when the absorption rates are very low and when the roots are exposed to comparatively large volumes of test solution. A thorough discussion of this phenomenon was presented in the earlier publication dealing with oats, and since the results obtained with buckwheat, if somewhat more pronounced, were similar to those obtained with oats, it is scarcely necessary here to emphasize further the importance of some adequate continuous flow system of solution

renewal during investigations of the nature here described, and for the growth of experimental plants in general where plant nutrition studies with culture solutions are involved.

The graphs of figure 1 represent the course of quantity absorption of nitrogen in the two forms only from the beginning of the blossoming period (18 days) to maturity; and without continuous flow of solution during the test intervals. Since the buckwheat plant enters the blossoming phase early in the life cycle and even before its vegetative development is in the advanced stages, it was interesting and important here to obtain a record of the course of nitrogen absorption during this early development from germination to flowering. To accomplish this it was necessary to develop a special technique involving a large number of plants to obtain absorption data with any degree of accuracy because of the small size and low quantity of nitrogen absorbed by individual plants. This is described in the following section.

Again in the data of table 1, as in the preliminary experiments with oats, some negative values are indicated for quantity absorption of nitrogen, both as NH_4 and NO_3 during several of the experimental periods. As previously stated (5) no adequate explanation of this phenomenon can here be offered. Suffice it to say that negative absorption (excretion) never occurred when the test solutions were continuously renewed during contact with the plant roots, as was done in the investigation of the *absorption series* described in the following sections.

The Absorption Series

Introductory. The plants of these series were harvested at the end of the experimental period with the termination of the absorption tests, and their dry weights obtained. This required a separate series of plants for the tests made during each experimental period. Seven such series of plants were grown and tested during the active life cycle. Each series consisted of six cultures which were conducted in duplicate. Tests were made at 4-day intervals up to the time the plants began to bloom at 18 days.

Because of their small size during the very early growth periods and the small quantities of nitrogen absorbed in a given time by individual seedlings, it was necessary to employ a large number of plants in a culture to provide for the removal by absorption of sufficient quantities of nitrogen from the culture solution to insure a fairly accurate measure by chemical analysis of the nitrogen so removed. To accomplish this, seeds were germinated between wet blotters in a moist chamber. A large number of strongly germinating seeds were then selected and placed on a germinating net tightly stretched over a small granite-ware pan in the manner described by Shive (3), the pan being filled with the culture solution. Here the plants were grown with continuous solution renewal up to the time when the absorption tests were begun. In preparation for the absorption test the old solution was drained from the pan, the plant

roots and pan were rinsed with distilled water and a measured quantity, 500 cc., of test solution was poured into the pan, the roots of the seedlings which protruded through the net being immersed. At the end of the test interval the net was removed from the pan, and the roots were rinsed with distilled water, which was retained with the solution for analysis. The total dry weight value of the plants from each pan was divided by the number of seedlings in the pan and multiplied by 3 to obtain the average weight of three plants, which in all cases is taken to represent the number of plants per culture upon which all calculations are based. Plants to be tested after the age of 14 days were mounted in paraffined cork stoppers as described by Tottingham (6), three plants to each culture, and their absorption was determined in the manner previously described (5).

The modified Tottingham solution $T_1R_3C_3$ (1) which contained the four salts KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$, and $(NH_4)_2SO_4$ in the following volume molecular proportions 0.0021, 0.0043, 0.0071, and 0.0042, respectively, and with approximately equal proportions of nitrogen in the two forms, NO_3, and NH_4, was used as the growth medium for the plants and also as the test solution during each of the seven experimental periods.

Throughout the test intervals the roots of the plants of each culture were immersed in a fixed quantity, 500 cc., of solution which was continuously renewed by being passed through the culture vessel at a constant rate of flow 1,000 cc. of new solution during the respective test interval. The total quantity, 1,500 cc., of solution to which the roots of a culture were exposed during the test interval was used for the chemical analyses to determine the quantities of nitrogen removed from the solution by the plants.

The results of the chemical analyses were calculated on the quantity basis of nitrogen absorbed in the two forms NH_4 and NO_3 per culture, of three plants, per hour, as well as on the rate basis of nitrogen absorbed in the two forms per gram of dry plant material per hour. The results obtained by the two methods of calculation are presented and discussed separately.

Absorption of nitrogen per culture per hour. In table 2 are presented the data dealing with quantities of nitrogen in the two forms in milligrams per culture per hour absorbed from culture solutions by buckwheat plants of seven different series. This table is constructed in precisely the same manner as table 1, but as previously stated, data are shown in each horizontal section for six instead of twelve test intervals. Thus each of the values marked *average* in the table represents the final average value of 24 determinations involving 12 cultures and 36 plants. These final average values only will be considered in the discussion, and they are plotted to form the graphs of figure 2.

It will be observed that the graph representing the march of quantity absorption of nitrogen as NH_4 in milligrams per culture per hour, begins with a low value at the 2-day period and rapidly rises to a maximum at 18 days, which

TABLE 2

Quantity absorption of nitrogen as NH_4, NO_3, and total nitrogen per culture per hour by buckwheat plants during absorption intervals ranging in time from 8 to 48 hours, at different stages of development throughout the active life cycle

AGE OF PLANTS	TEST INTERVALS	QUANTITY OF NITROGEN PER CULTURE ABSORBED					
		As NH_4		As NO_3		Total nitrogen	
		During test interval	Per hour	During test interval	Per hour	During test interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2	8	1.168	0.146	0.000	0.000	1.168	0.146
	16	1.763	0.110	0.000	0.000	1.763	0.110
	28	2.820	0.118	0.000	0.000	2.820	0.118
	32	3.327	0.104	0.006	0.000	3.333	0.104
	40	4.164	0.104	0.011	0.000	4.175	0.104
	48	5.045	0.105	0.011	0.000	5.056	0.105
Average			0 115		0.000		0.115
6	8	1.765	0.221	0.281	0.035	2.046	0.256
	16	3.198	0.200	0.446	0.028	3.644	0.228
	24	5.084	0.212	0.681	0.029	5.775	0.241
	32	7.006	0.219	0 815	0.025	7.821	0.244
	40	8.195	0.205	0.991	0.025	9.186	0 230
	48	8.944	0.186	1.261	0.026	10.205	0.212
Average			0.207		0.028		0.235
10	8	3.265	0.408	0.475	0.059	3.740	0.467
	16	6.368	0.399	0.594	0.037	6.976	0.436
	24	8.635	0 360	0.892	0.037	9.527	0.397
	32	11.309	0 353	1.247	0.039	12.556	0.392
	40	14.129	0.353	1.930	0.048	16.059	0.401
	48	17.395	0 362	2.078	0.043	19.473	0.402
Average			0.373		0.044		0.417
14	8	5.699	0.712	0.593	0.074	6.292	0.786
	16	8.786	0.549	1.247	0 078	10.033	0.627
	24	11.161	0.465	1.544	0 064	12.705	0.529
	32	13.595	0.425	1.900	0.059	15.495	0.484
	40	15 317	0.383	1.900	0.048	17.217	0.431
	48	19 098	0 398	1.900	0.040	20 998	0.438
Average			0.489		0.060		0.549
18	8	5.646	0.706	0.540	0.068	6.186	0.774
	16	8.139	0.509	2.130	0.133	10.269	0.642
	24	11.159	0.465	5.390	0.225	16.549	0.690
	32	17.885	0.559	5.390	0.168	23 275	0.727
	40	18.380	0.460	8.793	0.220	27.173	0.680
	48	20.144	0.420	9.860	0.205	30.004	0.625
Average			0.520		0.170		0.690

TABLE 2—*Concluded*

AGE OF PLANTS	TEST INTERVALS	QUANTITY OF NITROGEN PER CULTURE ABSORBED					
		As NH_4		As NO_3		Total nitrogen	
		During test interval	Per hour	During test interval	Per hour	During test interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
38	8	0.360	0.045	5.433	0.679	5.793	0.724
	16	1.190	0.074	9.377	0.586	10.567	0.660
	24	1.659	0.069	11.676	0.487	13.335	0.556
	32	2.547	0.080	13 518	0.422	16.065	0.502
	40	2.835	0.071	15.588	0.390	18.423	0.461
	48	3.411	0.071	16 743	0.349	20 154	0.420
Average			0.068		0.486		0.514
58	8	0 479	0.060	2.340	0.293	2.819	0.353
	16	1.031	0.064	5.408	0 338	6 439	0 402
	24	1.104	0.046	5.334	0.222	6.438	0 268
	32	1.178	0.037	6.288	0 197	7.466	0.234
	40	1.325	0.033	8.046	0.201	9.371	0.234
	48	1.472	0.031	9.072	0.189	10.544	0.220
Average			0.045		0.240		0.285

marks the beginning of the blossoming period. Thereafter the graph takes a steep downward slope and shows very low values for quantity absorption of nitrogen as NH_4 after the plants had passed through the blossoming stage.

The graph showing the march of quantity absorption of nitrogen as NO_3 in milligrams per cultuer per hour, starts from zero at the 2-day period, shows very low values during the early development of the plants up to the beginning of the blossoming period, after which the graph rises rapidly to a maximum point corresponding to a period considerably after the plants had passed the reproductive phase of development. Thereafter the graph takes a gradual downward slope, indicating continuously declining values as the plants approach maturity.

The course of the graph representing quantity absorption of total nitrogen shows a very steep upward slope to a maximum point which is determined by the absorption of nitrogen as NH_4 and corresponds to the maximum point in the graph representing the absorption of nitrogen as NH_4. Following the maximum point the graph takes a continuously downward slope, its course in the late phases of the life cycle being determined by the absorption of nitrogen as NO_3.

A comparison of the graphs of figure 2 with the corresponding graphs representing quantity absorption of nitrogen by the oat plant under like experimental conditions, brings out some interesting similarities as well as some significant differences between the behavior of the two species with respect to

the quantity absorption of nitrogen in the two forms here considered. The main point of similarity in the two species lies in the fact that both species utilize NH_4-nitrogen as the main source of supply of this element during the early stages of development, and NO_3-nitrogen as the main source of supply during late stages of growth. Both species show the period of maximum quantity absorption of total nitrogen during the blossoming period, but in the buckwheat series this point corresponds to, and is determined by, the point of

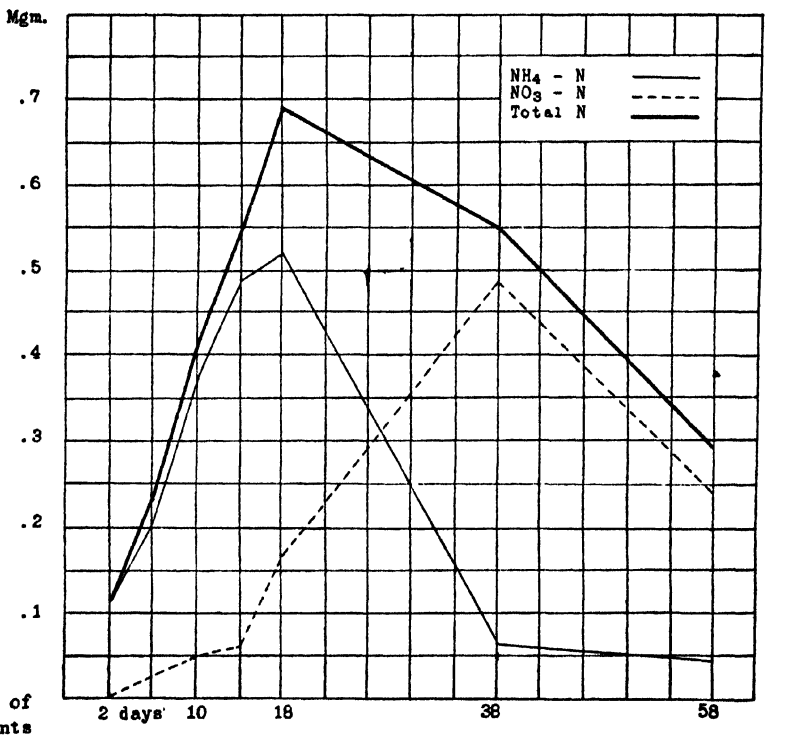

FIG. 2. GRAPHS REPRESENTING QUANTITY ABSORPTION OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN IN MILLIGRAMS PER CULTURE PER HOUR AT DIFFERENT PERIODS THROUGHOUT THE ACTIVE LIFE CYCLE OF THE BUCKWHEAT PLANT

maximum quantity absorption of NH_4-nitrogen; whereas in the oats series it corresponds to, and is determined by, the point of maximum absorption of NO_3-nitrogen.

In the buckwheat series, maximum quantity absorption NH_4-nitrogen occurs during the blossoming period, in the oats series it occurs at a much earlier stage. In the buckwheat series, maximum quantity absorption of NO_3-nitrogen occurs some time after the plants have passed through the blossoming period, in the oats series it occurs during the blossoming period. Thus from a comparison of the graphs representing the march of quantity absorption

of nitrogen in these two species it is apparent that quantity absorption of NH_4-nitrogen plays a much more pronounced rôle in the buckwheat series than it does in the oats series, and for more than half the active life cycle it is the predominating factor in determining quantity absorption of total nitrogen. On the other hand, quantity absorption of NO_3-nitrogen plays a much more pronounced rôle in the oats series than it does in the buckwheat series, and during nearly two-thirds of the active life cycle of the oat plant it is the predominating factor in determining quantity absorption of total nitrogen in this species, when the two forms of nitrogen are simultaneously present in the culture solutions in approximately equal proportions.

Absorption of nitrogen per gram of dry plant material per hour (rate absorption). The data relating to absorption rates calculated on the basis of nitrogen in the different forms absorbed per gram of dry plant material per hour, are presented in table 3. As before, only the final average values are considered in the discussion of these data. The final average values representing nitrogen absorbed as NH_4 and NO_3 in milligrams per gram of dry plant material per hour are designated the *NH_4-rate* and the *NO_3-rate*, respectively, for the sake of convenience. The final average rate values as given in table 3 were plotted to form the graphs of figure 3.

The graph representing the course of the NH_4-rate during the active life cycle begins by indicating high values even at the 2-day period, but the values increase to a maximum point at the 10-day period. Thereafter the graph takes a steep downward slope to a very low average value at the 38-day period. After the 38-day period the NH_4-rates are practically negligible.

The graph showing the course of the NO_3-rate begins by indicating a zero value at the 2-day period. The graph rises to a maximum at the 18-day period, which marks the beginning of the blossoming stage, and indicates values which are only slightly below the maximum throughout the entire reproductive period. After this, there is a very gradual decline with approaching maturity. However, it will be observed that the maximum NO_3-rate is relatively very low compared with the maximum NH_4-rate and occurs at a later period in the life cycle.

The graph representing the average rates of total nitrogen absorption is determined through the first half of the growth cycle by the NH_4-rates, and therefore takes the form of the NH_4-rate graph during these periods. It is only during the later phases of growth that the NO_4-rates exceed the NH_4-rates and only during that portion of the cycle when the rates of total nitrogen absorption are relatively very low.

A comparison of the rate graphs of figure 3 with the corresponding ones previously published (5) representing the course of the rates of nitrogen absorption by the oat plant, shows a marked similarity between the NH_4-rate graphs for the two species. Both indicate very high maximum values occurring during the early growth phases and very low values on approaching maturity. Likewise, the NO_3-rate graphs of the two species show remarkable

TABLE 3

Rate absorption of nitrogen as NH_4, NO_3, and total nitrogen per gram of dry plant material per hour by buckwheat plants during absorption intervals ranging in time from 8 hours to 48 hours, at different stages of development throughout the active life cycle

AGE OF PLANTS	TEST INTERVALS	DRY WEIGHT PER CULTURE (3 PLANTS)	ACTUAL NITROGEN ABSORBED PER GRAM DRY TISSUE PER HOUR		
			NH_4-N	NO_3-N	Total N
days	*hours*	*gm.*	*mgm.*	*mgm.*	*mgm.*
2	8	0.043	3.395	0.000	3.395
	16	0.045	2.444	0.000	2.444
	24	0.041	2.878	0.000	2.878
	32	0.046	2.261	0 000	2.261
	40	0.045	2 311	0.000	2.311
	48	0.046	2 283	0.000	2.283
Average		0.044	2.595	0.000	2.595
6	8	0 061	3 623	0.574	4.197
	16	0 059	3 390	0 475	3.865
	24	0.060	3.533	0.483	4.016
	32	0.065	3.369	0.385	3.574
	40	0.070	2.929	0.357	3.286
	48	0 075	2 480	0.347	2.827
Average		0.065	3.221	0.437	3.658
10	8	0.112	3.643	0.527	4.170
	16	0.105	3.800	0.352	4.152
	24	0.111	3.243	0.333	3 576
	32	0.114	3.096	0.342	3.438
	40	0.112	3.152	0 429	3.581
	48	0.113	3.204	0.381	3 585
Average		0.111	3.356	0 394	3.750
14	8	0.164	4.341	0 451	4.729
	16	0.150	3.660	0.520	4.180
	24	0.165	2.818	0.388	3.206
	32	0.180	2.361	0.328	2 689
	40	0.199	1 925	0.241	2.166
	48	0.191	2.084	0.209	2.293
Average		0.175	2.865	0.356	3.221
18	8	0.292	2.418	0.233	2.651
	16	0.300	1.697	0.443	2.140
	24	0.274	1.697	0.821	2.518
	32	0.308	1.815	0.545	2.360
	40	0.294	1.565	0.748	2.313
	48	0.310	1.355	0.661	2.016
Average		0.296	1.758	0.575	2.333

TABLE 3—*Concluded*

AGE OF PLANTS	TEST INTERVALS	DRY WEIGHT PER CULTURE (3 PLANTS)	ACTUAL NITROGEN ABSORBED PER GRAM DRY TISSUE PER HOUR		
			NH_4-N	NO_3-N	Total N
days	*hours*	*gm.*	*mgm.*	*mgm.*	*mgm.*
38	8	1 050	0.043	0.647	0.690
	16	1.123	0.066	0 523	0.589
	24	1 086	0.064	0 448	0.512
	32	1.152	0 069	0.366	0.435
	40	0 992	0.072	0.393	0.465
	48	1.078	0 066	0.324	0 390
Average		1 080	0.063	0.451	0.514
58	8	3.648	0.016	0.080	0.096
	16	3 726	0.017	0.091	0.108
	24	3.410	0.013	0 065	0.078
	32	3 406	0.011	0 058	0.069
	40	3.563	0.009	0.056	0 065
	48	3.485	0.009	0.054	0.063
Average		3 540	0.013	0.067	0.080

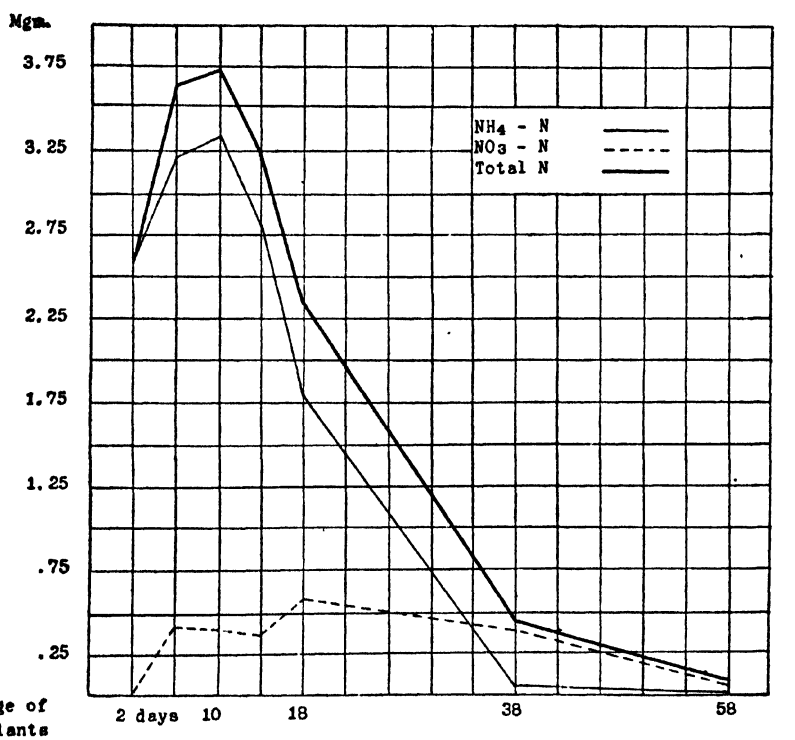

FIG. 3. GRAPHS REPRESENTING RATES OF ABSORPTION OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN IN MILLIGRAMS PER GRAM OF DRY PLANT MATERIAL, AT DIFFERENT PERIODS THROUGHOUT THE ACTIVE LIFE CYCLE OF THE BUCKWHEAT PLANT

agreement in that both begin with low values and reach their maximum points during the blossoming period. The comparison further shows that in the buckwheat series the NH_4-rate plays a greater rôle in the rate of total nitrogen absorption than it does in the oats series, and during more than half of the active life cycle of the buckwheat plant it is the predominating factor determining the rates of total nitrogen absorption. On the other hand, the NO_3-rate plays a more prominent rôle in the oats series than it does in the buckwheat series, and during more than half the active life cycle of the oat plant it is the predominating factor in determining the rate of total nitrogen absorption.

SUMMARY

Quantitative studies were made of the absorption of nitrogen by the buckwheat plant from culture solutions containing approximately equal proportions of nitrogen as NH_4 and NO_3, at various stages in the active life cycle of the plants. The main results of the investigations may be summarized briefly as follows:

Quantity absorption (milligrams per culture per hour) of nitrogen as NH_4 is at its maximum at the beginning of the blossoming period, declines rapidly after blossoming, and is low during the late stages of growth.

Quantity absorption of nitrogen as NO_3 is very low up to the beginning of the blossoming period, increases rapidly during the blossoming period, and reaches a maximum after the blossoming period, then declines rapidly.

Quantity absorption of total nitrogen attains a maximum point which corresponds to the point of maximum absorption of nitrogen as NH_4. This occurs at the beginning of the flowering period.

Rate absorption (milligrams per gram of dry plant tissue per hour) of nitrogen as NH_4 begins with high values and reaches a maximum early in the life cycle, and shows low values only during the late stages in the growth cycle.

Rate absorption of nitrogen as NO_3 is without value at a very early stage in the cycle, reaches a maximum during the blossoming period, then declines very slowly with approaching maturity.

The maximum NH_4-rate is approximately six times the maximum NO_3-rate.

Rate absorption of total nitrogen attains a maximum point which corresponds to, and is determined by, the point of the maximum NH_4-rate.

The NH_4-rate plays a much more prominent rôle in nitrogen absorption by the buckwheat plant than does the NO_3-rate, and during the greater part of the active life cycle it is the predominating factor in determining the rate of total nitrogen absorption. The NO_3 rates exceed the NH_4 rates only in the late stages of the growth cycle, when the rates of total nitrogen absorption are very low.

A comparison of buckwheat with oats, relative to nitrogen absorption, shows that NH_4-nitrogen absorption plays a much more prominent rôle in buckwheat than it does in oats, and NO_3-nitrogen absorption plays a much more pronounced rôle in oats than it does in buckwheat.

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THE INFLUENCE OF LEGUME VERSUS NON-LEGUME CROPS ON THE MICROBIOLOGICAL ACTIVITIES IN THE SOIL: II. NITRIFICATION AND CELLULOSE DECOMPOSITION[1]

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A previous publication (2) from this experiment station has considered the influence of legume versus non-legume crops on the numbers of microörganisms in the soil of an experimental field, and on the distribution of nitrogen-fixing activity over the field. The present report considers nitrification and cellulose decomposition in the soil of the same field.

Waksman (7), after a series of studies of nitrification in field soils, stated that his results indicate that studies of nitrification can yield information for the differentiation of soil fertility as well as can the determination of numbers of microörganisms. Waksman and Starkey (8) stated that the carbon dioxide evolved from soils, both without and with addition of small amounts of organic matter, can be used in grading the soils on a basis of their fertility, as well as can the determination of numbers of microörganisms and nitrification in soils.

EXPERIMENTAL

The field from which the soil used in the present experiments was taken has been described previously (2). A review of that description is given here.

The field is divided from north to south into four strips, each of which is 2 rods wide and 12 rods long. During the seasons when legumes are planted the strip on the west side of the field is planted with a legume crop, the strip next to the east is planted with a non-legume crop, then follow in order a strip planted with the same legume crop as the first and a fourth strip with the same non-legume crop as the second. These strips are designated by the crops planted thereon as: Legume West, Non-legume West, Legume East, and Non-legume East.

The field is further divided from west to east into strips which are 2 rods wide and 8 rods long each. These transverse strips are numbered from 5 to 10 inclusive. Strips 1 to 4 inclusive are not in use.

The division of the field into longitudinal and transverse strips results in 24 plots, each 2 rods square.

Each season the field is treated uniformly over its whole area with sulfate of potash and with superphosphate. The liming of the soil is described in the aforementioned previous publication (2), and in an outline from Morse given in the following. The nitrogen treatment differs with respect to the numbered strips. In past seasons strip 5 has been treated with sodium nitrate; strip 6, with ammonium sulfate until 1922 and none since; strip 8, with ammonium sulfate; and strip 10, with dry ground fish. Strips 7 and 9 are check strips with no nitrogen treatment.

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Professor F. W. Morse of the Department of Plant and Animal Chemistry of this Experiment Station, who is in charge of the experimental field, has furnished information concerning the treatment of the field and the crops planted since the period covered by the experiments already published (2). Professor Morse's statement follows:

1929. Nitrogen omitted from strips 5, 8, and 10. All plots fertilized with sulfate of potash and superphosphate as usual.
Grass and clover continued as crops from seeding of previous year.
1930. Fertilized as usual with sulfate of potash and superphosphate on all plots. Nitrogen applied to plots 5, 8, and 10.
Japanese millet seeded on all plots.
1931. 2,000 pounds ground limestone per acre applied to all plots, and sulfate of potash and superphosphate applied as usual. Nitrogen omitted from strips 5, 8, and 10.
Non-legume areas seeded with oats; legume areas seeded with mixture of oats and Canada peas. The oats are used to support the peas.

Representative samples of the soil from each plot of the field were taken in the spring of 1931 just before the field was plowed, and again in the fall just after the crop was harvested. Nitrification, cellulose decomposition, and nitrogen fixation were studied in both spring and fall samples.

Nitrification

The technic employed for the study of nitrification was essentially the same as that described by Waksman (6). The soil was dried sufficiently to permit screening through a 20-mesh sieve. The moisture content of the soil was determined in order to obtain desired quantities of the soils in terms of air-dried soil. The nitrate content of the soil as taken from the field was determined. Then three experiments were prepared: The first experiment consisted of 100-gm. quantities of soil with water added to, and maintained at, 60 per cent of saturation. This experiment was designed to study the nitrification of the organic matter present in the soil as taken from the field. The second experiment consisted of 100-gm. quantities of soil with sufficient ammonium sulfate added to supply 50 mgm. of nitrogen in 100 gm. of soil. Water was added to, and maintained at 60 per cent of saturation. The third experiment consisted of 100-gm. quantities of soil to which 1 gm. of dried blood (12 per cent N) per 100 gm. of soil was added. Water was added to, and maintained at, 60 per cent of saturation.

The experiments were set up in fruit jars of 1-pint capacity, and incubated for 4 weeks at 30°C. At the end of the incubation period the nitrate-nitrogen in the different jars was determined.

The procedure for the determination of nitrates was as follows:

The whole content of a jar (100 gm. of soil) was put into a large-mouth bottle with 5 gm. of calcium hydroxide (made by adding distilled water to lumps of calcium oxide until a fine white powder is obtained). After the calcium hydroxide had been mixed with the soil 400 cc. of distilled water was added. The calcium hydroxide was added to remove color from the

filtrate (5). After the water had been added the bottles were tightly stoppered and shaken for 10 minutes in a mechanical shaker. The entire content of a bottle was poured onto a filter paper in a funnel. The filtrate was poured back into the funnel several times until the filtrate was clear. After filtration was completed an aliquot portion of the filtrate was evaporated to dryness and the nitrate content determined by the phenoldisulfonic acid method as employed by Davis (3).

The results of the nitrification studies are shown in tables 1 to 4 inclusive. Variations in the quantities of nitrate are observed in all of the tables but the

TABLE 1

Nitrate in soil as taken from the field

Mgm. NO_3 in 100 gm. soil

STRIP NUMBER	SPRING				FALL			
	L. W.*	N L W.	L E.	N. L E	L W	N L W	L E	N. L. E.
10	7 1	8.8	11.0	6 2	4 4	12.7	8.0	8.8
9	7.1	9.7	6.2	15 1	5.3	9.7	9.7	8 8
8	8.8	9.7	6.2	8 8	6.2	8.8	8 0	8.0
7	10.6	6.2	11.5	9 7	5.3	4 4	8 8	6 2
6	7.1	7.9	7 1	8 8	8 8	8 0	7.1	8 0
5	5.3	7.9	7.1	8 8	7.1	4.4	9 7	7 1

* L. W. = Legume—West; N. L. W. = Non-legume—West; L. E. = Legume—East; N. L. E. = Non-legume—East.

TABLE 2

Nitrification of organic matter of the soil as taken from the field

Mgm. NO_3 in 100 gm. soil

STRIP NUMBER	SPRING				FALL			
	L W.	N L W.	L E	N. L E	L. W.	N L W	L E	N. L. E.
10	19 4	20 3	21.1	19 7	23 9	24 8	20 4	13.3
9	23 8	21 1	18 5	22.9	23.0	17.7	28 4	15 9
8	19.4	20.3	18.5	21.1	25.7	15.9	18 6	21 2
7	16 7	17 4	19 7	19 7	21.2	19 5	19.5	18.6
6	19.4	18.5	19 7	19 7	21.2	16.8	20 4	17.7
5	18 5	18.5	16.7	20 3	21.2	16.8	17.7	17.7

variations do not appear to be related either to the crop or to the soil treatment. There is no indication that the presence of legumes or non-legumes exerted any influence on the nitrifying activity of the soil. Dried blood yielded more nitrate than either the ammonium sulfate or the native organic matter of the soil. There was no apparent effect of season, the nitrification in each of the four experiments indicating that the nitrifying power of soil samples collected in the spring and those collected in the fall were comparable.

Cellulose decomposition

Soils employed for the study of cellulose decomposition were taken from the same samples as those studied for nitrification. They represented samples collected in the spring and samples collected in the fall. The soil was dried sufficiently to permit screening through a 20-mesh sieve. The moisture content of the soil was determined. Then sufficient soil was taken to give 100 gm. of air-dried soil. One gram of finely ground oat straw was thoroughly mixed with each 100-gm. portion of soil. Water was added to 60 per cent of satura-

TABLE 3

Nitrification of ammonium sulfate

Mgm. NO_3 in 100 gm. soil with 50 mgm. N as $(NH_4)_2SO_4$

STRIP NUMBER	SPRING				FALL			
	L. W.	N. L. W.	L. E.	N. L. E.	L W.	N. L. W.	L. E.	N. L. E.
10	106.3	159.5	141.8	154.1	146.2	150 6	110 7	128.5
9	150.6	132.9	119.6	150.1	146 2	106.3	110 7	128.5
8	141.7	132.9	146.2	154.1	128.5	93.0	101 9	124.0
7	132.9	132 9	106.3	146.2	110.7	119 6	128 5	101.9
6	128.5	115.2	141.8	146.2	101.9	115.2	146.2	88.6
5	110.8	115.2	119.6	146.2	124 0	93.0	101 9	106 3

TABLE 4

Nitrification of dried blood

Mgm. NO_3 in 100 gm. soil with 1 per cent dried blood (12 per cent N)

STRIP NUMBER	SPRING				FALL			
	L. W.	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
10	137.3	310.1	296.8	350.0	239 2	248.1	225.9	256.9
9	197.4	252.5	224.0	310.1	256.9	256.9	171.6	168.3
8	251.5	314.5	236.7	332.3	261.4	256.9	256.9	270.2
7	292.4	336.7	281.6	314.5	234.8	292.4	256.9	248.1
6	261.4	354.4	305.6	314.5	252.5	239.2	274.7	261.4
5	287.9	345.3	292.4	310.1	265.8	274.7	243 6	248.1

tion and maintained at that concentration for the duration of the incubation period. The experiments were set up in 500-cc. Erlenmeyer flasks which were closed with rubber stoppers containing inlet and outlet tubes to permit aeration The flasks were incubated at 26°C. until the evolution of carbon dioxide had dropped to a constant level, a period of 12 days. The evolution of carbon dioxide was employed as a measure of the rate of cellulose decomposition. An aeration method described by Waksman and Starkey (8) was employed for determining carbon dioxide. The air from the culture flasks was drawn through 0.25 *N* barium hydroxide, the excess barium hydroxide being titrated back with 0.25 *N* oxalic acid. Phenolphthalein was used as an indicator.

TABLE 5

Carbon dioxide evolution from soil + 1 per cent oat straw

Mgm. CO_2 per 100 gm. of soil, after 12 days

STRIP NUMBER	SPRING				FALL			
	L. W.	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
10	371	409	336	322	568	522	467	435
9	347	353	314	358	328	507	533	469
8	394	383	386	363	471	461	508	457
7	395	361	366	339	493	472	482	512
6	396	350	372	402	525	452	546	531
5	397	378	377	362	492	396	512	441

TABLE 6

*Nitrogen fixation**

Mgm. nitrogen increase per 50 cc. Ashby's solution from 1.0 gm., 0.1 gm., and 0.01 gm. quantities of soil

STRIP NUMBER	SOIL	SPRING				FALL			
		L. W.	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
	gm.								
10	1.0	+++	+++	+++	+++	+++	+++	+++	+++
	0.1	0	0	+	++	++	+	+	++
	0.01	0	0	0	+	+	0	0	++
9	1.0	+++	+++	+++	+++	+++	+++	+++	+++
	0.1	0	0	++	+++	0	+	+	+++
	0.01	0	0	+	++	0	+	+	+++
8	1.0	+++	++	+++	+++	+++	+++	+++	+++
	0.1	0	+	+++	+++	+	+	++	++
	0.01	0	0	0	0	0	+	+	+
7	1.0	+++	+++	++	+++	+++	+++	+++	+++
	0.1	+	0	+	+	++	+	+	++
	0.01	0	0	0	+	+	+	+	+
6	1.0	++	++	++	+++	++	+++	+++	+++
	0.1	+	+	+	+	++	++	+	+
	0.01	0	0	0	0	++	+	+	+
5	1.0	++	+++	++	+++	+++	+++	+++	+++
	0.1	0	0	+	+++	0	+	+	++
	0.01	0	0	0	+	0	+	+	+

* +++ = Over 2 mgm. nitrogen per culture.
++ = 1 to 2 mgm. nitrogen per culture.
+ = Less than 1 mgm. nitrogen per culture.
0 = No nitrogen increase.

The results of the cellulose decomposition studies are shown in table 5. There is no indication that the process was influenced by the presence of legume or non-legume crops. In that respect the studies agree with the nitrification studies. Carbon dioxide evolution was greater in the soil samples collected in the fall than in those collected in the spring, indicating that the decomposition of cellulose was more active in the fall. This increase of activity appeared to be general over the entire experimental field.

Nitrogen fixation

In order to compare the functions of nitrification and cellulose decomposition with that of nitrogen fixation in a given season, nitrogen fixation studies were made with the soil samples collected in the spring and those collected in the fall. The procedure was the same as that used in the previously published (2) study of this series. The results were in agreement with those previously published, in that the legumes or non-legumes did not appear to influence nitrogen fixation by the soil microörganisms in cultures. Since the results obtained in the present study were comparable with those published earlier (2) no tables are included in this paper.

A study of the distribution of nitrogen-fixing bacteria in the soil of the various plots was made by a dilution method. Blake culture bottles were prepared, each containing 50 cc. of Ashby's solution (1). One gram, 0.1 gm. and 0.01 gm. respectively from each soil sample were inoculated into culture bottles. After two weeks of incubation the nitrogen increase was determined by the Kjeldahl method. The results are shown in table 6. It will be observed that the east half of the field, the legume-east and non-legume-east strips, showed more samples of soil giving some nitrogen increase in the 0.01 gm. inoculations than was obtained with soils from the west half of the field. The only reason that can be given is that the field slopes downward from west to east with the result that the moisture retention was probably greater in the east half of the field. The results do not warrant any assumption that the presence of legumes or non-legumes had any influence on the amount of nitrogen fixed.

SUMMARY

Nitrification of ammonium sulfate, dried blood, and the native organic matter by the soil from different plots of an experimental field did not appear to be influenced by the presence of legume or non-legume crops in the field. The results obtained from soil samples collected in the spring and in the fall did not differ significantly

Cellulose (oat straw) decomposition in soil from the different plots of the field, as indicated by the evolution of carbon dioxide, did not appear to be influenced by the presence of legume or non-legume crops in the field. The evolution of carbon dioxide was greater in the samples collected in the fall than in samples collected in the spring, indicating that cellulose decomposition was more active in the fall.

Nitrogen fixation studies with the soil from the different plots of the field gave results comparable with those previously published (2). Nitrogen fixation did not appear to be influenced by the presence of legume or non-legume crops in the field.

Nitrification, cellulose decomposition, and nitrogen fixation studied gave results which were in agreement, in that there was apparently no influence exerted by the presence of legume or non-legume crops in the field.

The results of chemical analyses of crops taken from the field, and of the soil from the field, have been published by Morse (4).

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